CORRELATION BETWEEN PLASMA FIBRINOGEN AND GLYCOSYLATED HEMOGLOBIN IN TYPE II DIABETES MELLITUS PATIENTS

Dissertation submitted to

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY,

CHENNAI – 600032

In partial fulfillment of the requirement for the Degree of

Doctor of Medicine in Physiology (Branch V)

M.D. (PHYSIOLOGY)

APRIL 2015

DEPARTMENT OF PHYSIOLOGY

COIMBATORE MEDICAL COLLEGE

COIMBATORE – 14

CERTIFICATE

This dissertation entitled **"CORRELATION BETWEEN PLASMA FIBRINOGEN AND GLYCOSYLATED HEMOGLOBIN IN TYPE II DIABETES MELLITUS PATIENTS"** is submitted to The Tamil Nadu Dr. M.G. R Medical University, Chennai, in partial fulfillment of regulations for the award of M.D. Degree in Physiology in the examinations to be held during April 2015.

This dissertation is a record of fresh work done by the candidate **Dr. S.BHUVANESWARI ,** during the course of the study (2012-2015).

This work was carried out by the candidate herself under my supervision.

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Coimbatore – 14.

DECLARATION

I Dr.S.Bhuvaneswari solemnly declare that the dissertation entitled **"CORRELATION BETWEEN PLASMA FIBRINOGEN AND GLYCOSYLATED HEMOGLOBIN IN TYPE II DIABETES MELLITUS PATIENTS"** was done by me at Coimbatore Medical College, during the period from August 2013 to June 2014. Under the guidance and supervision of **Dr.N.Neelambikai M.D.,** Professor and HOD, Department of Physiology, Coimbatore Medical College, Coimbatore. This dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University towards the partial fulfillment of the requirement for the award of M.D. Degree (Branch - V) in Physiology.

I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

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Course : M.D PHYSIOLOGY.

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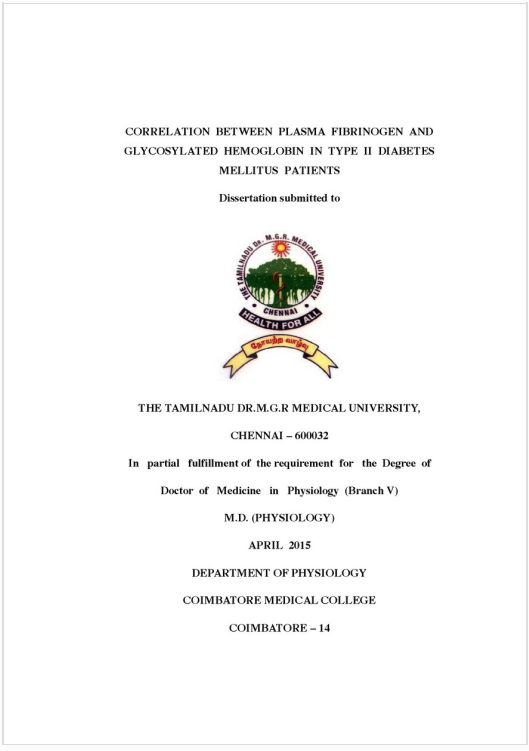

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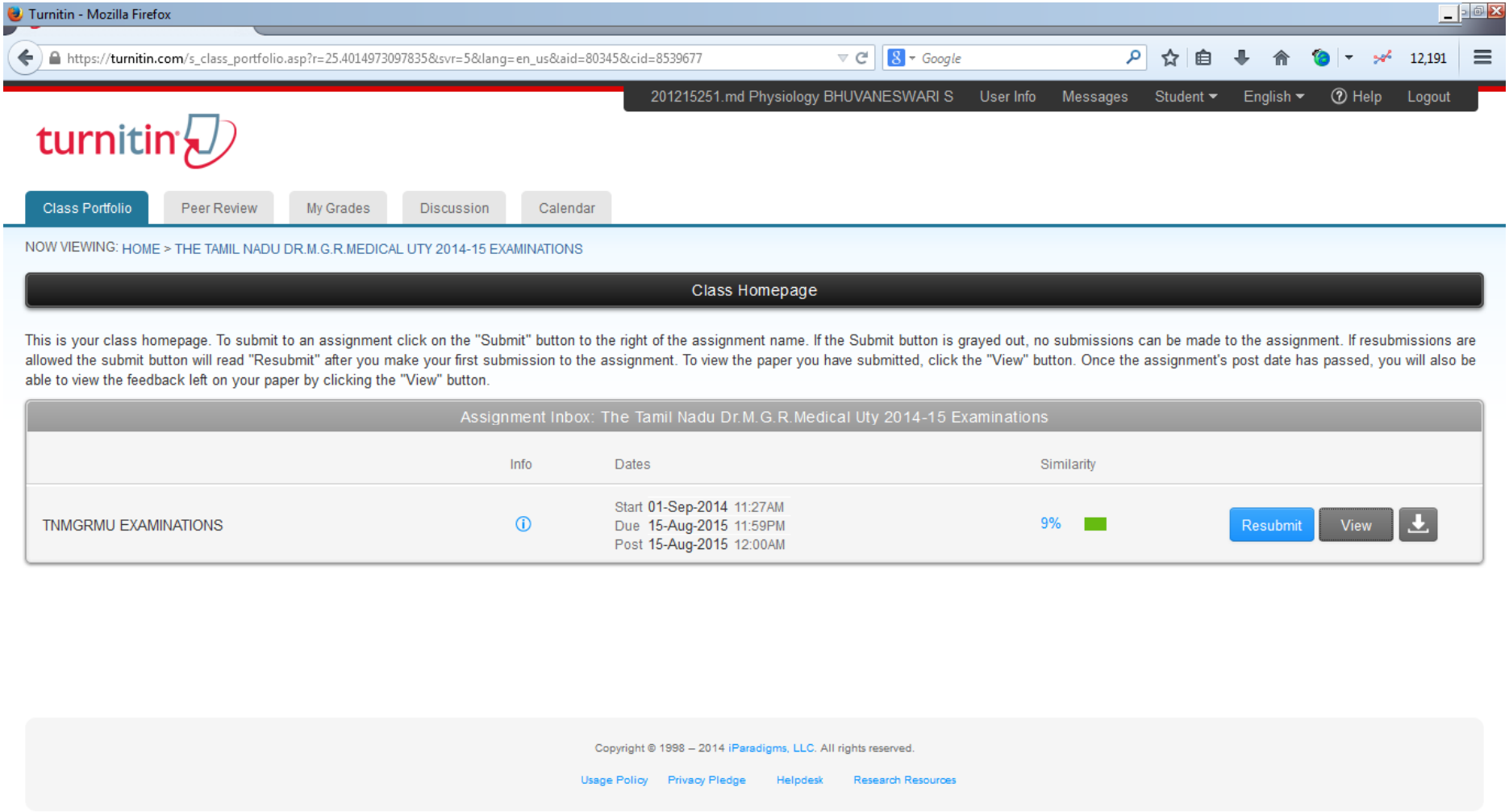
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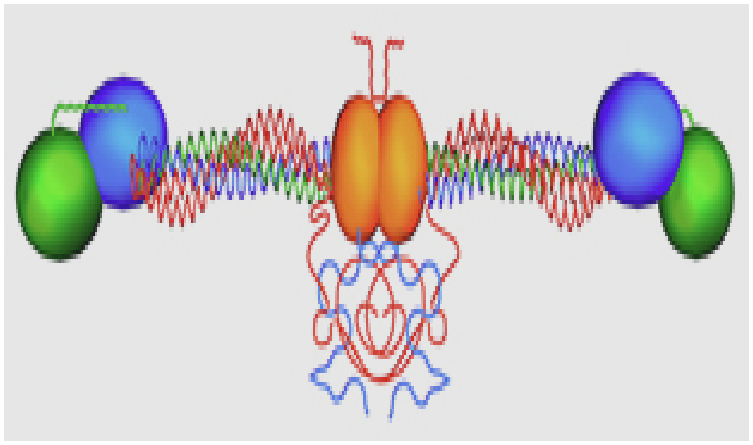


CONTENTS

ABBREVIATIONS USED IN THE STUDY

- FPA — FIBRINOPEPTIDE A
- FPB — FIBRINOPEPTIDE B
- GP IIb-IIIa — GLYCOPROTEIN IIb-IIIa
- HMWK — HIGH MOLECULAR WEIGHT KININOGEN
- Hb A1C — HEMOGLOBIN A 1C
- PAI-1 — PLASMINOGEN ACTIVATOR INHIBITOR – 1
- AGE — ADVANCED GLYCATION END PRODUCTS
- t-PA — TISSUE PLASMINOGEN ACTIVATOR
- u-PA — UROKINASE PLASMINOGEN ACTIVATOR
- GLUT — GLUCOSE TRANSPORTER
- LDL — LOW DENSITY LIPOPROTEINS
- VLDL — VERY LOW DENSITY LIPOPROTEINS
- ADA — AMERICAN DIABETES ASSOCIATION
- NCEP — NATIONAL CHOLESTEROL EDUCATION PROJECT
- UKPDS — UNITED KINGDOM PROSPECTIVE DIABETES STUDY
- IRS — INSULIN RECEPTOR SUBSTRATE

CORRELATION BETWEEN PLASMA FIBRINOGEN AND GLYCOSYLATED HEMOGLOBIN IN TYPE II DIABETES MELLITUS PATIENTS

Background: Type II diabetes mellitus occurs due to deficient insulin production in the setting of insulin resistance. In India, more than 62 million people are diagnosed as having diabetes. The risk of developing coronary artery disease is 2-4 fold high in diabetics and this excess risk was not fully explained by classical risk factors like smoking, hypertension and dyslipidemia. Fibrinogen in its high level involved in all stages of atherogenesis and considered as an important cardiovascular risk factor.

Aim and objectives: To compare the plasma fibrinogen level between type II diabetics and healthy individuals. To correlate the plasma fibrinogen level with HbA1c value in type II diabetic patients.

Materials and methods: This is cross sectional type of study. 75 type II diabetic patients of age between 40-60 years taken as study group. 75 age and sex matched healthy individuals taken as control group. Study subjects were selected from the outpatient department of Diabetology, CMCH. Patients with history of systemic hypertension, smoking, vascular complications of diabetes, infectious and inflammatory conditions were excluded from the study. Blood sample was taken and analysed for plasma fibrinogen and HbA1c by turbidimetric immunoassay method.

Results: Statistical analysis was done by using student 't'test and pearson correlation coefficient. In the present study, mean plasma fibrinogen level was significantly increased ($p < 0.0001$) in diabetic patients (276.6 ± 87) when compared to control group (160.3 ± 49) . significantly increased ($p < 0.0001$) plasma fibrinogen level was observed in diabetics with

HbA1c of >7% (334.2±77) and also positively correlated with HbA1c value (r=0.8166).

Conclusion: In the present study , increased plasma fibrinogen level (upper limit of normal range) was observed in diabetic patients having HbA1c of more than 7%. It could be due to advanced glycation end products mediated endothelial injury, apoprotein - a and increased amount of glycosylated fibrinogen. Elevated plasma fibrinogen along with other blood clotting factors produce hypercoagulable state in diabetes mellitus. Increased plasma fibrinogen also involved in all stages of atherogenesis, which results in formation of occlusive thrombus. So the measurement of plasma fibrinogen is considered as an important tool to assess cardiovascular risk in diabetic patients.

Key words: diabetes, fibrinogen, HbA1c, cardiovascular risk factor.

INTRODUCTION

Diabetes mellitus is a chronic disorder of metabolic derangements sharing the common feature of hyperglycemia resulting from impaired action and/or reduced secretion of insulin [1]. It is rapidly achieving the potential epidemic status in India with more than 62 million people are diagnosed as having diabetes. In 2000, India has attained the top place with 31.7 million diabetic population followed by china with 20.8 million people and united states with 17.7 million people [2].

In 2010, the globally estimated population of diabetes mellitus was about 285 million, among them, 90% type II diabetics. In 2013, 381 million people were affected globally with prevalence expected to almost double by the year 2030[3,4]. Increasing prevalence of diabetes in developing countries like India is because of life style modifications like westernisation of diet, and reduced physical activity. Morbidity and mortality due to potential macrovascular and microvascular complications of diabetes are very high and it is responsible for major health care burden to the community[2].

In diabetes, peripheral neuropathy is accounting for about 24.6% of complications, which is followed by cardiovascular diseases (23.6%), nephropathy(21.1%), retinopathy (16.6%), and foot ulcers (5.5%) [5]. Risk of developing coronary artery disease is 2-4 fold higher in patients with diabetes than in non diabetic subjects[2].

According to WHO 2013, cardiovascular diseases are considered as most leading cause of death, worldwide. Among the cardivascular diseases, coronary artery disease is the leading one and accounting for 1.4 million deaths in the developed countries and 5.7 million deaths in the developing countries[6].

In 80% of diabetic patients death occur due to thrombotic events, among which 75% is being due to coronary artery diseases and remaining due to cerebrovascular accidents and peripheral vascular diseases. Occurrence of premature atherosclerosis and extensive vascular diseases is responsible for plaque rupture and subsequent thrombus formation leading onto cardiovascular events [7,8].

The Framingham heart study reported that, the prevalence of cardiovascular disease among diabetic patients have increased when compared to non diabetic subjects[9]. According to Silver et al[10], in patients with unstable angina, more number of diabetic

patients (94%) had ulcerated plaque in the affected coronary artery than non diabetic subjects (60%). Study done by Moreno et al[11], observed that atheroma of diabetic patients constitute large amount of lipid content, increased infiltration with macrophages, and thrombosis while compared to non diabetic subjects.

In diabetic subjects without previous history of myocardial infarction (MI), the risk of associated mortality due to MI is equal to that of non diabetic patients with previous history of MI. In 2002, Diabetes was named as coronary artery disease equivalent by NCEP report [12].

The classical risk factors responsible for cardiovascular diseases in diabetes are obesity, smoking, hypercholesterolemia, and hypertension, with the primary trigger being the hyperglycemia [1]. But the excess cardiovascular risk in diabetes was not fully explained by these classical factors. An analytical study reported that >50% of subjects with coronary artery disease had atleast one of the classic risk factors and about 20% of subjects had coronary artery disease without the presence of any classical risk factors. In addition to these classical risk factors, some other novel risk factors which include fibrinogen, C-reactive

protein, serum uric acid, homocysteine, LP(a) and tumor necrosis factor also were reported. These factors accelerate the thrombotic process and resulting in the cardiovascular complications[13].

Fibrinogen is a soluble glycoprotein present in the plasma and designated as first clotting factor in the blood coagulation cascade. In case of severe injury to the blood vessel, fibrinogen is converted into insoluble fibrin and forms a definite clot at the site of injury. Increased fibrinogen level in the plasma leads to increased the blood viscosity, endothelial injury and platelet aggregation, which plays a role in formation of atherosclerosis and subsequent thrombosis.

In 1980, Meade and colleagues, first found out the association between coronary artery diseases and high levels of plasma fibrinogen. Then numerous studies had proved the association between hemostatic parameters and atherosclerosis associated cardiovascular complications[13]. Luciana et al observed that plasma fibrinogen level was significantly elevated in patients with CAD when compared with normal healthy subjects. In their study, plasma fibrinogen level was correlated with the severity of CAD [15]. In patients with known coronary artery disease, strong correlation between plasma fibrinogen level and recurrence of MI

was observed. Significant association between plasma fibrinogen level and cerebrovascular diseases, occlusive peripheral arterial diseases was also observed in fewer studies [16].

Levels of plasma fibrinogen may vary with classical cardiovascular risk factors like diabetes mellitus, hypertension, smoking, obesity and dyslipidemia [17]. Several studies have demonstrated the association between diabetes mellitus and increased plasma fibrinogen level.

In the vascular system, primary protection against the thrombosis is intact endothelial layer of the vessel wall. This endothelium gets injured in case of diabetes by different mechanisms including, endothelial activation by advanced glycation end products (AGE) and Protein kinase-c (PKC) activation leading to altered basement membrane structure and vascular permeability. This endothelial injury predisposes prothrombotic state and accelerates the process of atherosclerosis in diabetic patients.

Prothrombotic state is associated with increased plasma levels of many coagulation factors including fibrinogen, factor VII, XI, XII, Kallikrein, factor VIII /vWF complex, impaired fibrinolysis by elevated PAI-1 and highly resistant nature of the

clot to the fibrinolysis[8]. Insulin deficiency associated metabolic stress in the liver can also causes increase in plasma fibrinogen especially in diabetes [18].

The elevated plasma fibrinogen is involved in early steps of plaque formation resulting in cardiovascular morbidity later. Fibrin molecules bind with oxidised LDL molecules and forming the atheromatous plaque. Then by forming occlusive thrombus at the site of plaque rupture, increased fibrinogen level is involved in the late cardiovascular diseases like MI, angina and stroke[19].

Various studies have shown that diabetic patients with poor glycemic control prone for high risk of cardiovascular morbidity and mortality. HbA1c levels has been utilised to assess the glycemic control in diabetic patients. Only fewer studies were carried out to know the correlation between plasma fibrinogen and glycemic control in diabetic patients. So this study has been done to evaluate the correlation between plasma fibrinogen level and glycosylated haemoglobin (HbA1c) in type II diabetes mellitus patients.

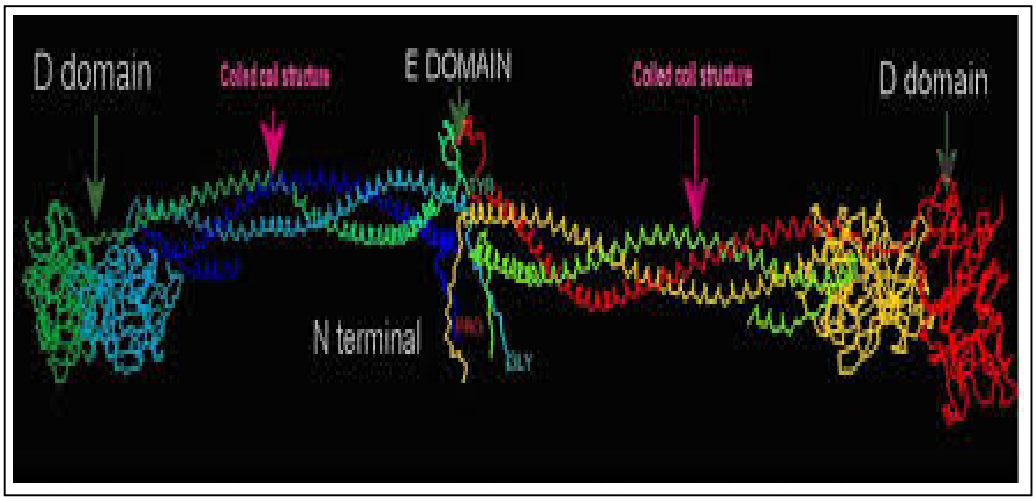
AIMS & OBJECTIVES

AIM AND OBJECTIVES

- To study the level of plasma fibrinogen in type II diabetes mellitus patients and healthy individuals.
- To compare the plasma fibrinogen level between type II diabetes mellitus patients and healthy individuals.
- To evaluate the correlation between plasma fibrinogen level and glycosylated haemoglobin in type II diabetes mellitus patients.
- To evaluate the association between plasma fibrinogen level and duration of diabetes in type II diabetes mellitus patients.

REVIEW OF LITERATURE

FIBRINOGEN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REVIEW OF LITERATURE

FIBRINOGEN

Fibrinogen is a multipotential plasma protein synthesized by the liver and is an essential component of blood coagulation system. It is also responsible for viscous nature of blood, hence determines the blood flow. Increased plasma level of fibrinogen alters vascular homeostasis by causing hypercoagulability. This in turn increases the risk of cardiovascular diseases like myocardial infarction, stroke and peripheral vascular diseases [20].

HISTORICAL REVIEW [21]

In 1686, Malphigi separated the fibrin fibers from clotted blood which is devoid of red blood cells and serum, then demonstrated those fibres by using a single lens microscope.

In 1771, a junior surgeon named William Hewson identified that clot formed from fluid portion of blood and he also observed the fast erythrocyte sedimentation in his patients with severe inflammatory pathology.

In 1801, Furcroy, named it as fibrin. He also demonstrated the soluble precursor of fibrin present in the plasma and it was termed "Fibrinogen" by Babington in 1830.

ANDREW BUCHANAN

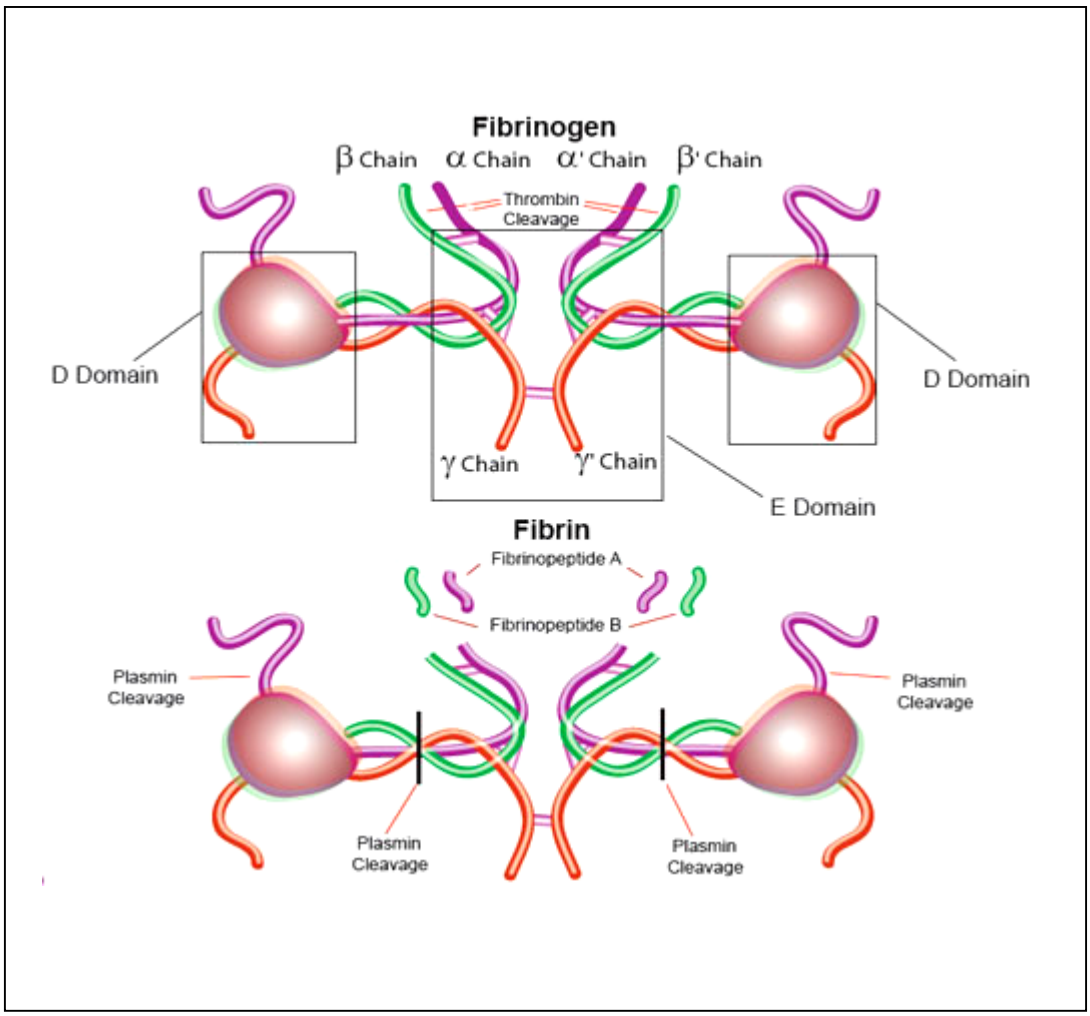
In 1836, Andrew Buchanan, a young surgeon working at Glasgow university described in detail about the fibrin by his experiments on fresh blood clot. He took the fresh blood clot in a linen cloth and by squeezing, separated fluid from it. When the squeezed out liquid was added to hydrocele fluid, it clotted.

In another experiment, he diluted the blood with water and allowed it to clot overnight. Then he squeezed the liquid from clotted blood and mixed it with hydrocele fluid. Fibrin present in the extracted fluid coagulated the hydrocele fluid. By these experiments he concluded that, fibrin of animal fluids exists in the form of solution.

In 1859, Denis, first separated the fibrinogen by salt precipitation technique. In 1879, Hammerston, obtained the pure form of fibrinogen by repeated salt precipitation technique.

In 1909, Mellanby who worked under the guidance of Alexander Schmidt, prepared fibrinogen by diluting plasma followed by its acidification with acetic acid. Hammersten found out that, fibrin is formed by reaction between fibrinogen and thrombin in 1879.

STRUCTURE OF FIBRINOGEN AND FIBRIN



Physiological Aspects of Fibrinogen[22,23]

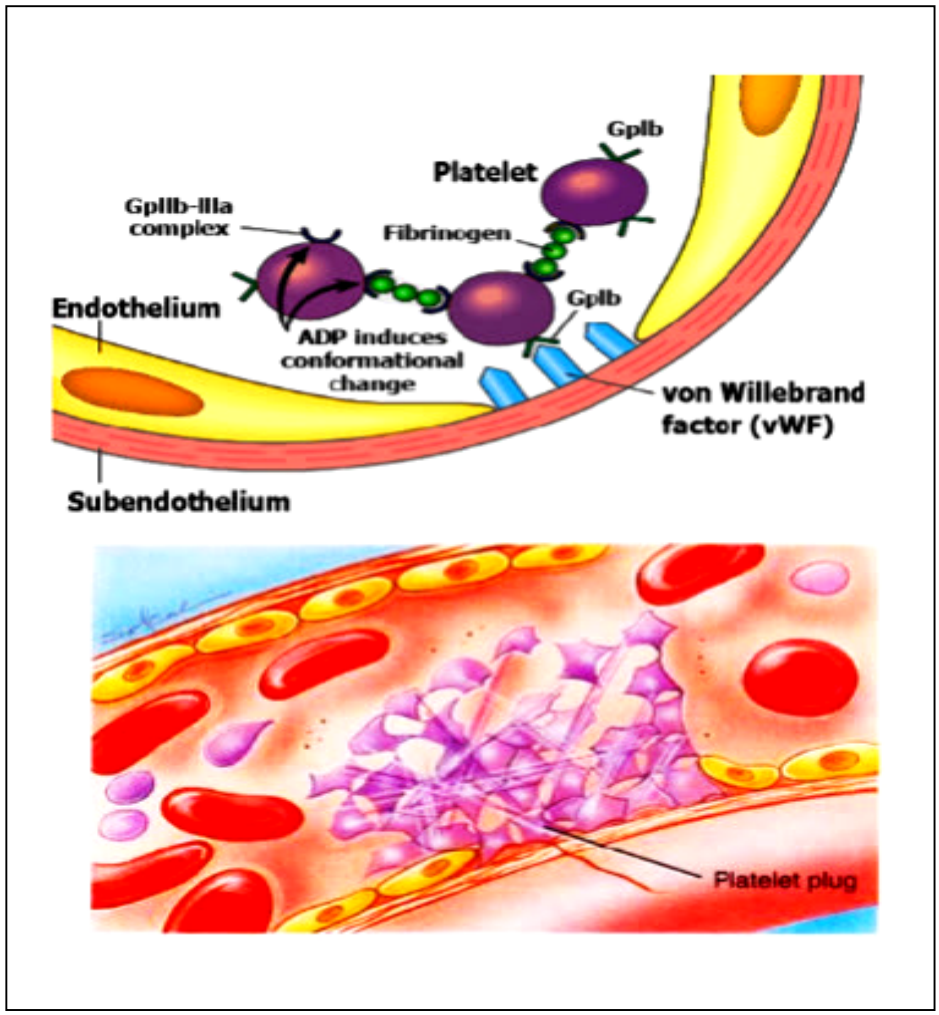
Fibrinogen is one of the major plasma protein present in the blood. It is a high molecular weight (340 kD) soluble glycoprotein that circulates in the plasma, as a precursor of insoluble fibrin molecule. It is synthesised mainly in the liver, and taken up by the platelets in smaller amount and stored in the granules, which is released during the process of platelet activation and aggregation. In response to inflammatory stimulus, it is also secreted by the lungs and intestinal epithelium.

Fibrinogen is made up of two symmetric subunits of 3 different polypeptide chains namely Aα, Bβ, γ. These polypeptide chains bind with one another by covalent disulfide linkages. Amino terminal ends of all 3 pairs of chains forming the central 'E' domain, whereas carboxy terminals of Bβ and γ chains

forming two peripheral 'D' domains. Amino terminal ends of α and β chains designated as fibrinopeptide A and B respectively. FPA and FPB act as repulsion force and prevent polymerisation of fibrinogen. Thrombin cleaves the two pairs of these peptides during the conversion of fibrinogen into fibrin.

Normal level of plasma fibrinogen is about 200-450 mg/dl[24]. Its level may vary from 100-700mg/dl [25]. The amount of

PRIMARY HEMOSTATIC PLUG FORMATION



fibrinogen synthesised is between 1.7 to 5 g per day with mean plasma level of about 250mg/dl. Half life is between 3-5 days. The minimum concentration of fibrinogen required for hemostasis is about 50-100mg/dl[26].

FUNCTIONS OF PLASMA FIBRINOGEN [25,27,28]

I. PRIMARY HEMOSTATIC PLUG FORMATION:

In daily life, numerous small endothelial damages occur in the very small blood vessels. These minute injuries are corrected by the platelet plug formation. Fibrinogen promotes platelet aggregation by binding with GP IIb-IIIa receptors present on the adjacent platelets leading to temporary hemostatic plug formation.

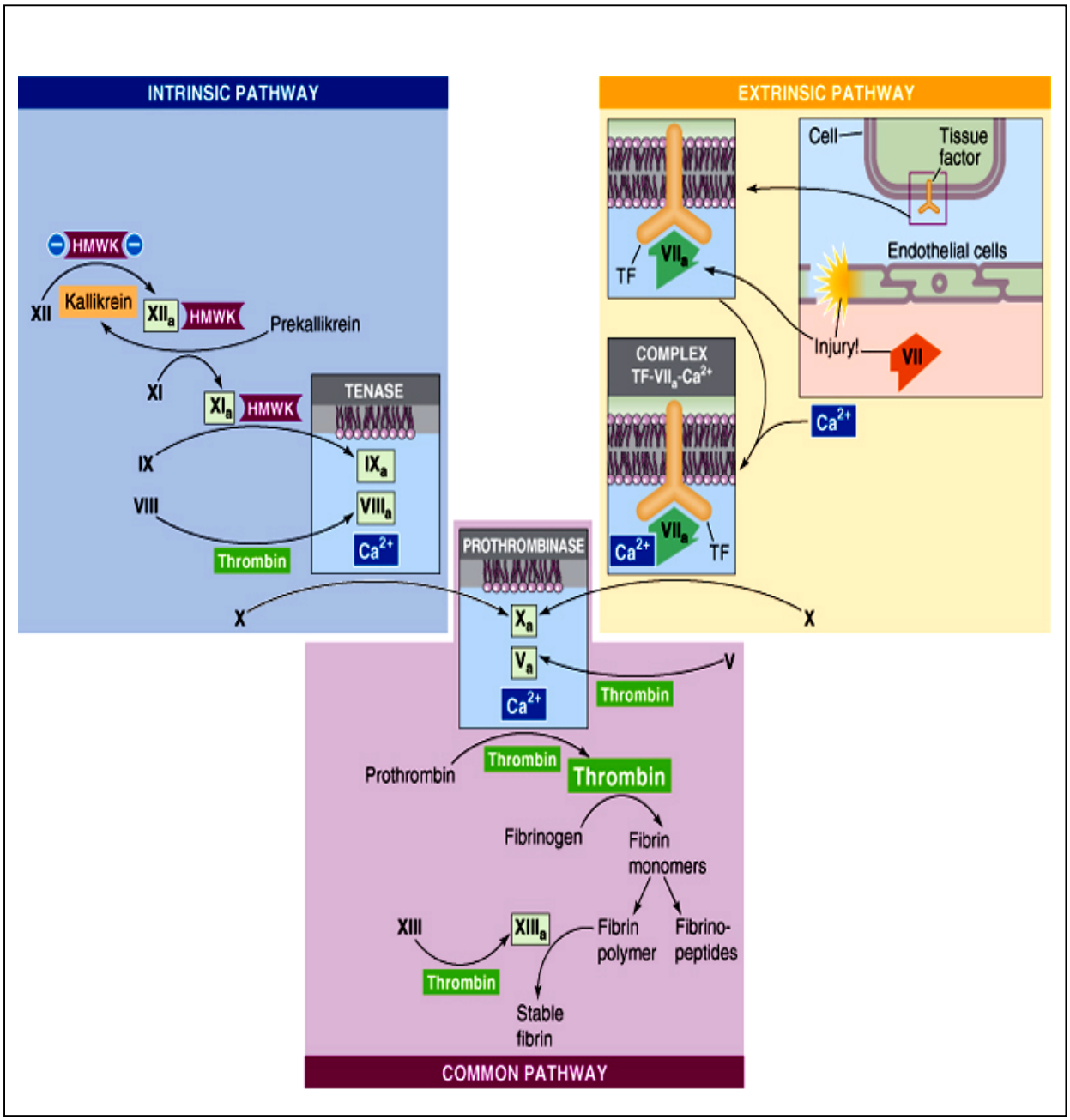
II. FORMATION OF DEFINITE FIBRIN CLOT:

In case of severe injury to the vessel wall, blood coagulation mechanism starts to develop within 15-20 seconds of injury and prevent further blood loss by forming fibrin clot.

BLOOD COAGULATION OCCURS IN 3 MAJOR STEPS:

- Formation of prothrombin activator complex by extrinsic and intrinsic pathway.
- Conversion of prothrombin into thrombin
- Conversion of fibrinogen into fibrin

COAGULATION PATHWAY



FORMATION OF PROTHROMBIN ACTIVATOR COMPLEX

Intrinsic Pathway :

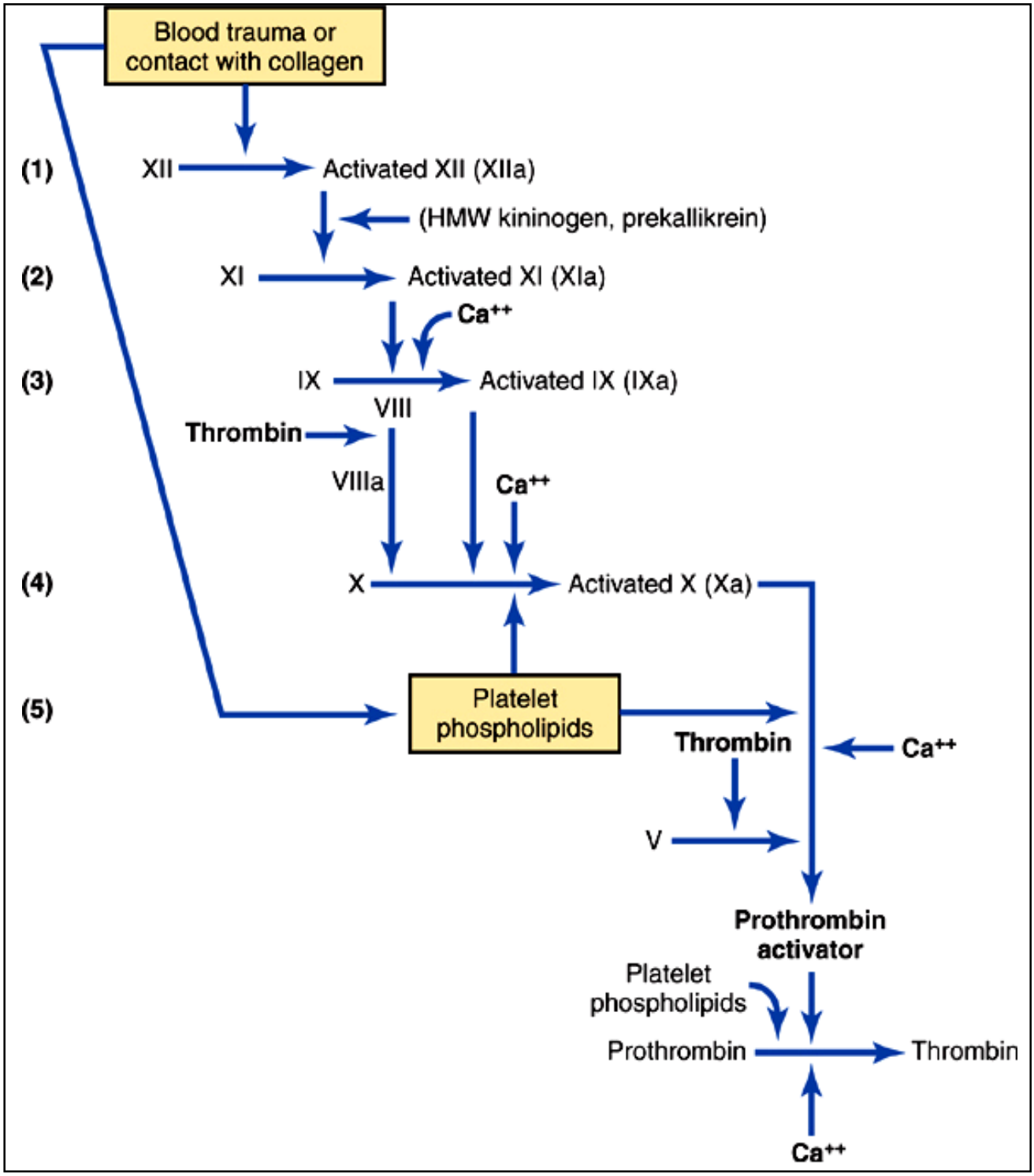
This mechanism starts with exposure of blood to subendothelial collagen tissue through damaged vessel wall or by injury to blood. Initial step is the activation of factor

XII by its contact with collagen tissue. In the presence of HMWK and prekallikrein, activated factor XII converts the factor XI into activated factor XI. Factor XIa acts as enzyme and is involved in the activation of factor IX. In the presence of platelet phospholipid and platelet factor 3, factor IXa converts the factor X into activated factor X.

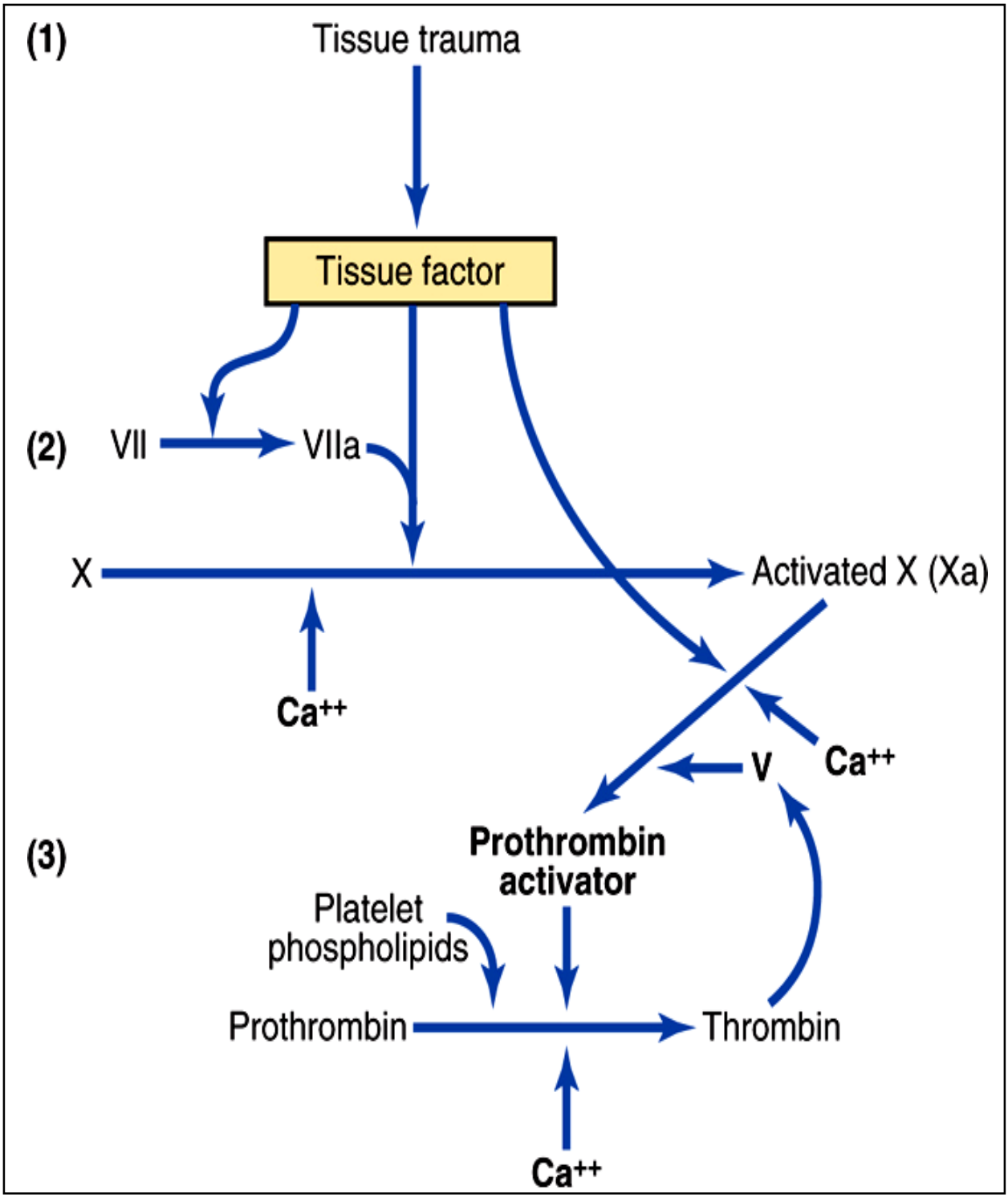
Extrinsic Pathway:

It starts with the injury to the vessel wall or surrounding tissues. Injured tissues releases thromboplastin. Along with factor VII and Ca^{2+} the tissue factor converts factor X into Xa.

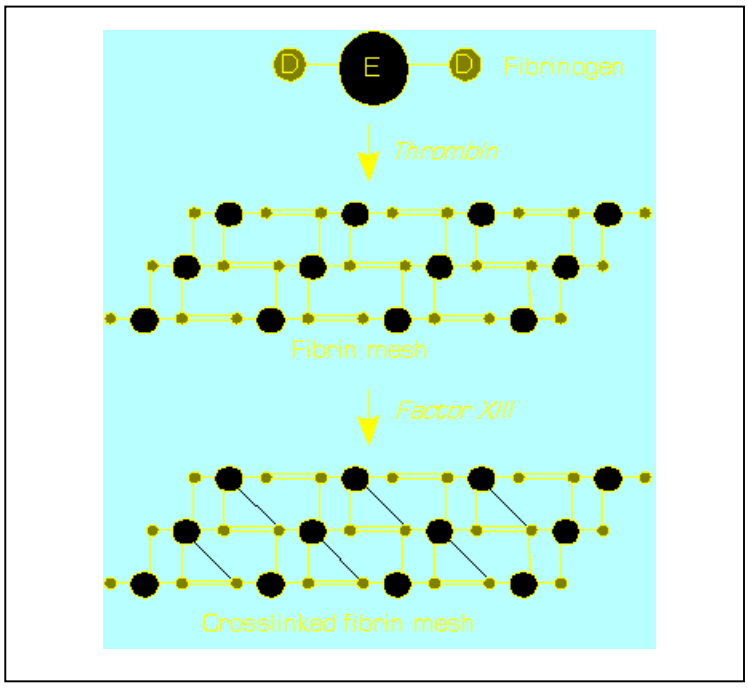
INTRINSIC PATHWAY



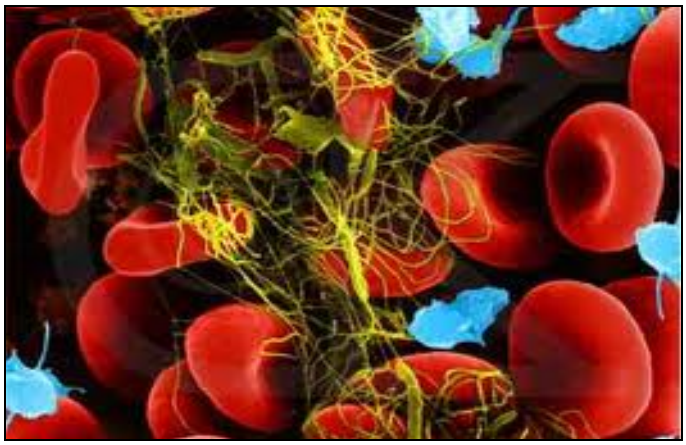
EXTRINSIC PATHWAY



CONVERSION OF FIBRINOGEN INTO FIBRIN



FIBRIN CLOT



Finally the “Prothrombin activator complex” is formed which contains factor Va, factor Xa, Ca2+ and platelet phospholipid.

Conversion of Prothrombin into Thrombin

Factor Xa having enzymatic activity converts the prothrombin into thrombin , and factor Va accelerating this process.

Conversion of Fibrinogen into Fibrin:

It is the final step of blood coagulation mediated by thrombin which acts enzymatically and converts fibrinogen into fibrin. It occurs in 3 steps.

Proteolysis of Fibrinogen:

Soluble fibrinogen contains one central ‘E’ domain and two peripheral ‘D’ domains. Thrombin binds with fibrinogen on its central domain, then proteolytically cleaves the bond between fibrinopeptides and aminoterminal ends of polypeptide chains. fibrinopeptide A and B are released from Aα and Bβ chain respectively forms fibrin monomer.

Various forms of fibrin monomers are ‘desA fibrin’ formed after rapid removal of fibrinopeptide A, ‘desAB fibrin’ produced after the release of both fibrinopeptide A and B and ‘desB fibrin’ formed rarely after removal of Fibrinopeptide B.

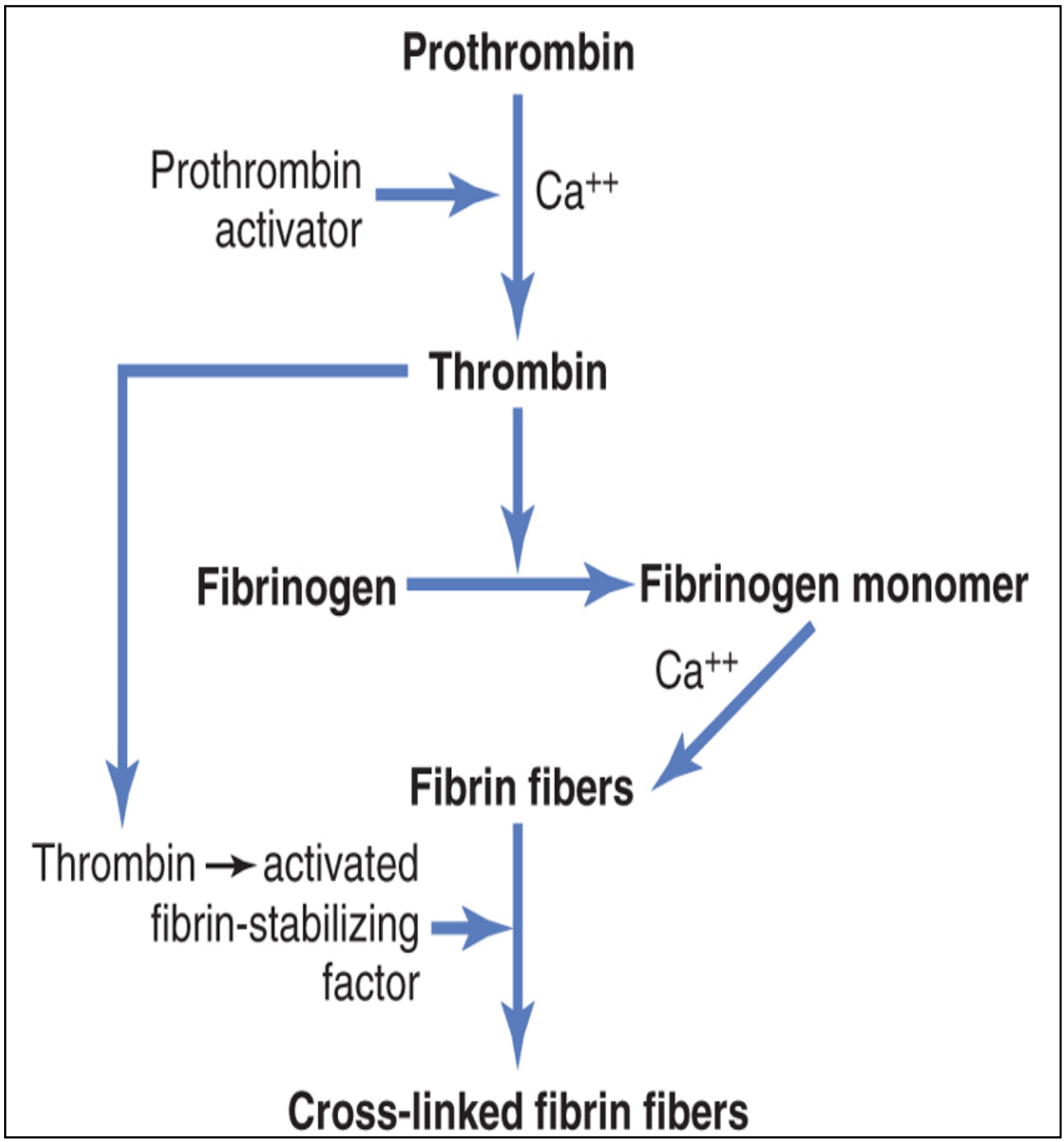
Polymerisation of fibrin monomer:

Fibrin monomers join laterally to form fibrin oligomer. whereas more fibrin monomers unite to form polymer called “Protofibril”. Many protofibrils join laterally and forms thick fibrin fibers. This fibrin polymerisation process is mediated by thrombin and factor XIIIa.

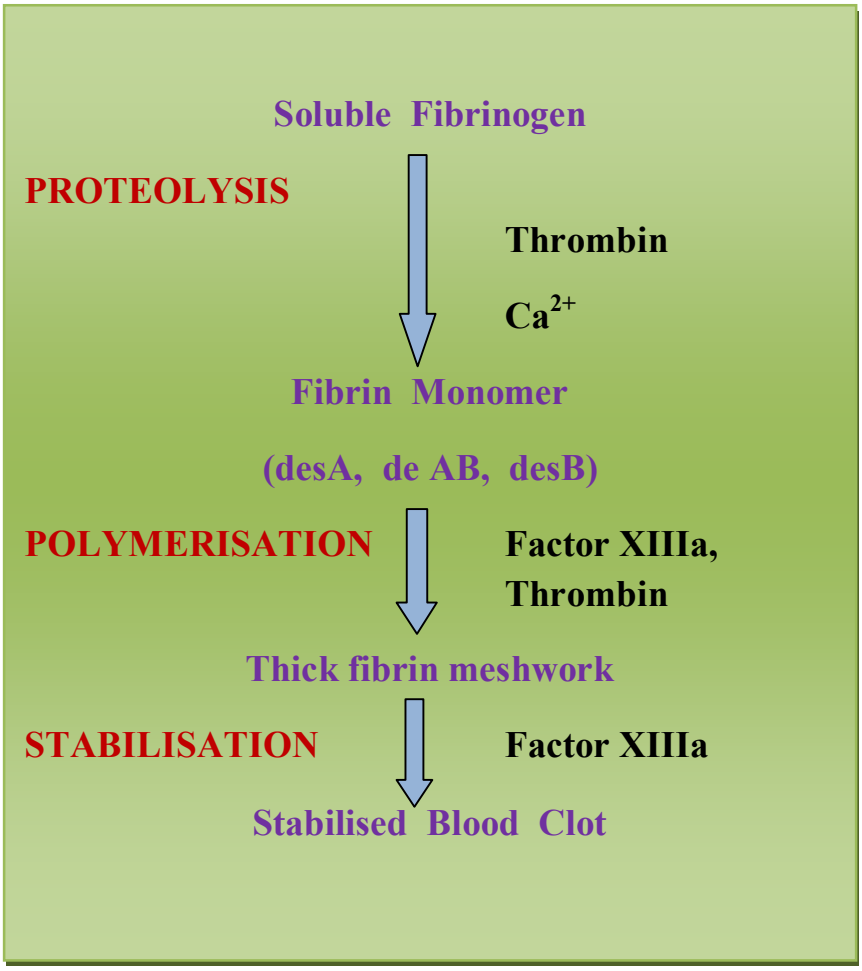
Stabilisation of fibrin polymer:

Factor XIIIa and Ca^{2+} mediates the covalent cross linking of fibrin polymers within the fibrin bundle. This covalent cross linkages between fibrin polymers strengthen the fibrin meshwork and stabilised blood clot is formed with entrapment of Red blood cells and Platelets inside the blood clot

CONVERSION OF FIBRINOGEN INTO FIBRIN



STEPS INVOLVED IN CONVERSION OF FIBRINOGEN INTO FIBRIN



III. BLOOD VISCOSITY [29]

It is a measure of intrinsic resistance to the blood flow. Blood viscosity is 1.6 times more than the water by the presence of plasma proteins like globulin and fibrinogen. Asymmetric elongated structure of Fibrinogen is responsible for the viscous nature of blood.

IV. ERYTHROCYTE SEDIMENTATION [30]

When the blood column is allowed to standstill in a tube, the rate at which red blood cells that settles down is determined by the amount of fibrinogen in the blood.

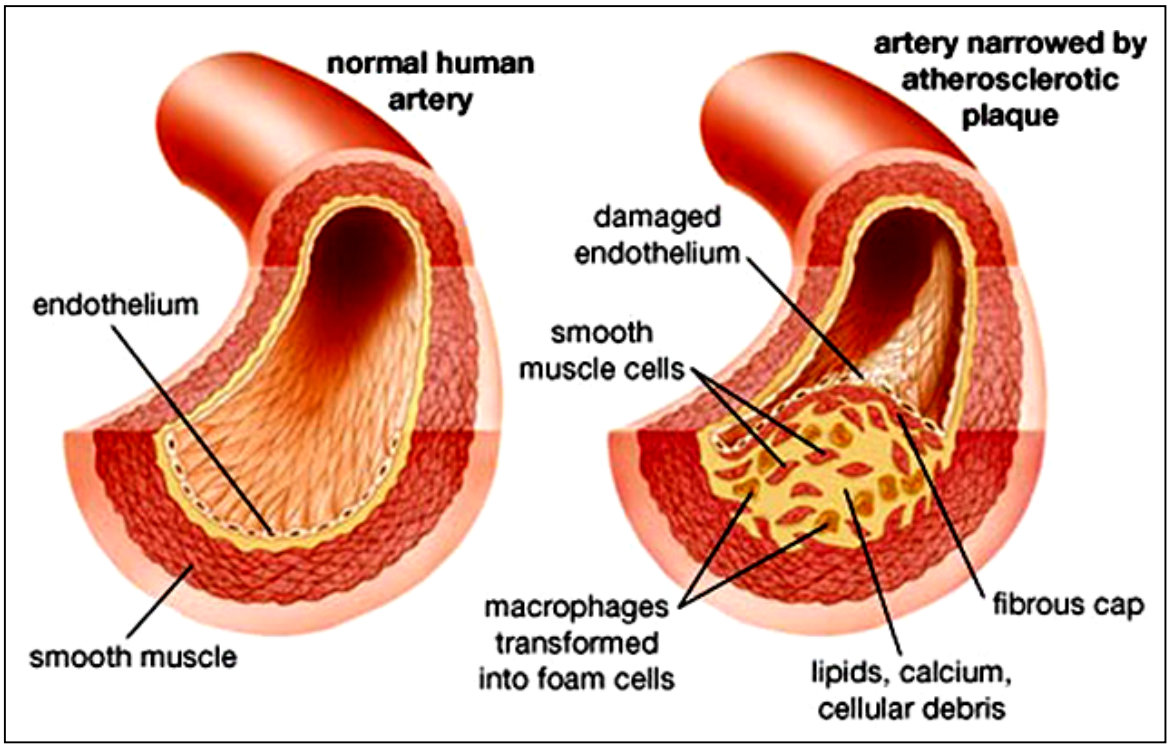
Red blood cells having negatively charged sialic acid molecules on their cell membrane which act as repulsion force separates the red blood cells from one another. This repulsion force is also called as zeta potential. Fibrinogen neutralises these zeta potential and makes the red blood cells to stick with each other by which it increases the rate of settling down of red blood cells .

CONDITIONS INCREASING FIBRINOGEN LEVEL

There are so many factors influencing plasma fibrinogen level. The factors contributing to increase in plasma fibrinogen level are conventional cardiovascular risk factors like diabetes mellitus, systemic hypertension, hypercholesterolemia, smoking and obesity.

Others are advancing age, menopause, malignancies, infections, inflammations and oral contraceptive pills intake.[41]

ATHEROSCLEROSIS



PATHOPHYSIOLOGIC ASPECTS OF FIBRINOGEN IN RELATION TO CARDIOVASCULAR DISEASES

Fibrinogen and its active product called fibrin play an important role in atherosclerosis and thrombosis by altering blood rheology, causing endothelial injury and promoting platelet aggregation [29].

ATHEROSCLEROSIS:

The intimal lesion known as "Atheroma" protrudes into the vessel lumen is the characteristic feature of atherosclerosis. This atheromatous plaque is made up of three portions.

1. Superficial fibrous cap contains collagen and smooth muscle cells.
2. Sides of fibrous cap, which is made up of smooth muscle cells and macrophages.
3. Necrotic core which is deep to fibrous cap contains lipoprotein (a) and low density lipoprotein, lipid laden macrophages called foam cells, dead cell debris, fibrin, microthrombus and other plasma proteins [31].

ROLE OF FIBRINOGEN IN ATHEROSCLEROSIS:

Increase in plasma fibrinogen level in turn increases blood viscosity. This change in viscosity increases the responsiveness of platelets to the subthreshold level of ADP, which is the important substance promoting platelet aggregation.

Normally tunica intima of arterial wall contains plasma proteins like lipoprotein (a), fibrinogen and low density lipoprotein. These proteins are also present in the atherosclerotic vessel wall and their concentration depends on plasma concentration [32].

In case of endothelial injuries, repairing processes are activated to maintain the vessel homeostasis. These processes include, platelet aggregation, movement of leukocytes across endothelial cell layer and proliferation of smooth muscles in the vessel wall. All these events occur in a co-ordinated manner by various cytokines, growth factors and adhesion proteins. Changes in any of these events by triggering factors like increased plasma fibrinogen during repair process lead onto lipid deposition, extra cellular matrix protein accumulation, cell death resulting in advanced atheromatous plaque formation [33].

Fibrinogen is a multipotential protein involved in all stages of atheromatous plaque development. On increased plasma fibrinogen level, more amount of fibrin is deposited in the tunica intima leading to endothelial injury and edema. The initial feature of early proliferative lesion is " Intimal edema". Fibrin acts as stimulant for smooth muscle migration into intima and its further proliferation.

Fibrin also continuously split to form fibrin degradation products. These FDPs have mitogenic activity and maintaining the fibrin induced smooth muscle cell proliferation. FDPs also stimulate collagen synthesis and alters endothelial permeability. Fibrin also bind lipoprotein (a) and low density lipoprotein with more affinity and promotes their accumulation and prevent their migration within the lesion [34,35].

Increased fibrinogen gets deposited in the vessel wall; fibrin molecules may form the mural thrombus on the intact surface of plaque, or deposit within the fibrous cap or in the lipid rich portion of atheromatous plaque or spread diffusely throughout the plaque. This may also be associated with decreased intimal fibrinolysis and reduced plasmin concentration in the blood as seen in cardiovascular diseases [20].

By binding with adjacent platelets , it promotes platelet aggregation, and by forming definite clot over the ruptured plaque involved in the dreadful events of atherothrombosis [19].

STUDIES RELATED TO FIBRINOGEN AND CARDIOVASCULAR DISEASES

Cardiovascular diseases including, coronary vascular diseases and cerebrovascular diseases, are nowadays the major cause of morbidity and mortality worldwide and responsible for 21.9% of total deaths. By the year 2030, this percentage value may increase to 26.3% [36] .

Conventional cardiovascular risk factors does not predict all cardiovascular events. In an analytical study of more than 1,20,000 persons with coronary artery disease, about 20% subjects have manifested coronary artery disease without the presence of conventional risk factors like systemic hypertension, diabetes, smoking and dyslipidemia. More than 50% subjects had only one of the above conventional risk factors.

These observations suggested the possibility of other risk factors in the occurrence of coronary artery disease and focussed the experimental research into the identification of novel risk factors like increased levels of plasma fibrinogen, WBC count, homocysteine, C reactive protein, uric acid and adiponectin levels[13].

Several risk factors like systemic hypertension, diabetes mellitus, dyslipidemia and smoking are associated with the pathogenesis of coronary atherosclerosis. Along with these conventional risk factors, more number of newer risk factors like increased lipoprotein-a, homocysteinemia, increased PAI-1, more number of reactive oxygen species, angiotensin converting enzyme polymorphism are also added in the pathogenesis of atherosclerosis.

Inflammatory products like C-reactive protein and fibrinogen are also responsible for atherosclerosis. These are highly sensitive and produced in response to inflammatory cytokine IL-6, which is the major factor inducing the synthesis of acute phase proteins According to Yuksel Cavusoglu et al [37], mean CRP and fibrinogen values were significantly high, whereas AT-III level was significantly low in patients with Coronary artery diseases while comparing with that of patients without Coronary artery diseases.

In 1987, The Framingham heart study observed statistically significant association present between plasma fibrinogen level and coronary artery disease. They selected 1315 subjects without coronary artery diseases. Over the study period of 12 years 165 men and 147 women subjects developed cardiovascular diseases. In both sexes, Cardiovascular disease risk is positively correlated with the high plasma fibrinogen level of more than 126 to 696 mg/dl. Plasma fibrinogen level was also significantly associated with other cardiovascular risk factors like systemic hypertension, obesity, smoking and diabetes mellitus. By this study, increased plasma fibrinogen level was considered as a predictor of cardiovascular diseases and suggested to be included in the cardiovascular risk profile[38].

Northwick park heart study: Meade et al, analysed the involvement of thrombotic factors in the development of coronary artery diseases. They have investigated 1511 male subjects of age between 40-64 years. Association between Coronary artery disease and factor VII activity, fibrinogen and cholesterol levels were 62%, 84%, 43% respectively. This indicated that the strong association was present between fibrinogen and Coronary artery diseases, especially in young adults than in old age in this study.

They have also observed strong association between Coronary artery disease and smoking may be mediated through increased plasma fibrinogen[39].

In another prospective epidemiological study, 2167 males and 941 females in the average age of 48 years were recruited and studied over the period of 29 years. During the follow up period, 231 males and 36 females died because of Coronary artery disease. Factor VII activity measured at the time of recruitment was strongly associated with mortality due to coronary artery disease in both sexes. Whereas the measured fibrinogen level was strongly correlated with mortality due to coronary artery disease in men but not in women. This correlation persists even after adjusting for confounding factors. This strong association also confirmed at the time of 6 year follow up measurement of fibrinogen level and factor VII activity . They have suggested that, the hemostatic factors were also involved in morbidity and mortality of coronary artery disease [40].

The major three mechanisms involved in the basis of pathogenesis of atherosclerosis are endothelial dysfunction, thrombosis and inflammation. Fibrinogen and its metabolites produce endothelial dysfunction. Fibrin accumulated in the intimal

layer of vessel wall binds with the fibronectin, which triggers the cell migration and adhesion. They also stimulate the mitogenesis, collagen synthesis, leukocyte migration and increase in capillary permeability.

Pro inflammatory cytokines like IL-6 and TNF-α released from the blood vessels, adipose tissue and cardiac myocytes inducing the synthesis of fibrinogen, which acts via prothrombotic mechanism involved in the formation of atheromatous plaque, the initial stage of coronary artery diseases. This indicates that the fibrinogen is important etiological factor responsible for coronary artery diseases rather than the byproduct of atheromatous plaque [41,42].

According to Shojaie et al, high levels of fibrinogen is considered as risk factor for premature coronary artery disease in persons of <55 years of age[43].

Green D et al observed that, high levels of fibrinogen involved in the subclinical atherosclerosis. They also found that, there was strong positive correlation present between high levels of fibrinogen in young adults and prevalence of coronary artery calcification and increased carotid artery intimal- medial thickness in middle age[44].

Framingham offspring study: James J. Stec et al concluded that, plasma fibrinogen level was increased in patients with cardiovascular diseases than those without CVD and increase in fibrinogen level also correlated with other conventional cardiovascular risk factors[17].

According to Amanda J lee, patients with history of systemic hypertension, stroke, diabetes mellitus and in persons with intermittent claudication, the mean plasma fibrinogen level was high while comparing with that of healthy subjects. In persons with myocardial infarction, angina, and in persons with family history of premature heart disease before the age of 60 years plasma fibrinogen level was significantly higher than the normal healthy subjects[45].

Gheye.S et al, observed that in Indian people significant association present between elevated plasma fibrinogen, abdominal obesity and coronary artery disease[46].

Luciana et al, also observed that plasma fibrinogen level was significantly elevated in patients with coronary artery disease while comparing with that of normal healthy subjects. The plasma fibrinogen level correlated with the severity of coronary artery disease [15].

Increasing incidence of Coronary artery disease in the young adults (CADY) suggests the involvement of non-conventional newer risk factors like thrombogenic factors, infections, inflammation and psychosocial factors[47].

In the study by Murat saruc et al, the levels of Protein-C, Protein-S were significantly reduced and plasma fibrinogen level was significantly elevated in patients with coronary artery disease than in controls. Significant changes in above parameters also present between patients with single vessel disease and in patients with double or triple vessel disease[48].

J stuart and co workers described that, Patients with severe lower limb atherosclerosis showed significant increase in platelet count, fibrinogen, neutrophilic leukocytosis, and defect in platelet function and fibrinolysis. These abnormalities are together called haematological stress syndrome seen in chronic vascular diseases and responding to formation of atherothrombosis [49].

Robert M. Corney et al, found that, in patients with coronary artery disease and depression, fibrinogen levels were in the high normal level. These elevated fibrinogen levels were in significant negative correlation with HRV parameters. Because of depression associated decreased parasympathetic activity, vagal

inhibition over the proinflammatory cytokines release became reduced, lead on to increased plasma fibrinogen level. This plasma fibrinogen inturn increases the risk of mortality in patients with coronary artery disease and depression [50].

The interpretation of Raja Babu Panwar and his co workers in their study is that, thrombogenic factors like, elevated plasma fibrinogen level, homocysteinemia, smoking, inadequate ingestion of fruits and vegetables and atherogenic factors like high fat intake, systemic hypertension, dyslipidemia (high LDL, low HDL, high triglycerides) were involved in the development of premature coronary artery disease [51].

In the cohort study done by A.R.Rudnika and co-workers, strong association was observed between plasma fibrinogen level and cardiovascular diseases like coronary artery disease and stroke even after adjustments made for all other risk factors [52].

De Maat MP et al described that, plasma fibrinogen level was significantly higher in patients with symptoms of angina while compared to healthy control group and there is difference in plasma levels of IL-6 between patients and healthy controls while no difference was observed in the CRP level[53].

Atherosclerosis Risk In Communities (ARIC) study:

plasma fibrinogen was increased in persons with prevalent cardiovascular diseases while compared to healthy subjects. It also positively correlated with stroke, intermittent claudication and carotid atherosclerosis[54].

TYPE II DIABETES MELLITUS

Definition: [55]

Diabetes mellitus refers to prolonged hyperglycemia resulting from group of metabolic dearrangements involving major nutrients like carbohydrate, lipids, and proteins caused by impaired insulin action with or without reduced insulin secretion.

Historical aspects :[55]

In ancient era, Sushruta, father of surgery gave an understandable view of diabetes in the form of etiological factors, clinical features and complications. He also introduced the rules of medical and surgical treatments for the diabetes. At that time diabetes was named as “Madhumeha” (means “rain of honey”), because of the sweet taste urine of patients with diabetes.

In 1st century AD, The Greek physician arateus of Cappadocia, coined the term “diabetes” means “siphon” that is liquefaction of flesh and bones into urine.

In 1674, Thomas Willis, professor at oxford rediscovered the sweety taste of urine of patients with diabetes by tasting that urine.

Cullen (1710-1790): He gave the full name diabetes mellitus by adding the term “mellitus” (‘mel’ means ‘honey’) with diabetes.

In 1788, Thomas Cowley found out that shrunken pancreas studded with stones in an autopsy of diabetic patient.

In 1798, John Rollo found out the relationship between hyperglycemia and diabetes mellitus.

In 1869, Paul langerhans, a german medical student described that different types of cells combined and presented as islets. These islets were spread throughout the pancreas with majority in the tail portion of pancreas. Later it was named as “Islets of langerhans”.

20 years later, role of pancreas in diabetes was demonstrated by Oscar Minkowski and Joseph Von Mering. They were surgically removed the pancreas in two dogs. On the next day both dogs developed fatal diabetes with polyuria. Then the dogs life were saved by placing fresh piece of pancreatic tissue underneath the skin.

DIAGNOSTIC CRITERIA FOR DIABETES MELLITUS:[1]

- Symptoms of diabetes mellitus with random blood sugar ≥200 mg/dl (or)
- Fasting plasma glucose (FPG) concentration ≥126 mg/dl (or)
- 2 hours plasma glucose concentration ≥200mg/dl during oral glucose tolerance test.

Most reliable method for diagnosis is measurement of FPG concentration.

CRITERIA FOR DIAGNOSIS OF DIABETES AND PRE DIABETES [56]

Test	Normo glycemia	IFG	IGT	Highrisk	Diabetes
FPG (mg/dl)	<100	100-125			≥126
2 hours PG(mg/dl)	<140		140-199		≥200
HbA_{1c} (%)				5.7-6.4	≥6.5

CHARLES.H.BEST AND FREDERICK.G.BANTING

PHYSIOLOGICAL BASIS OF INSULIN SECRETION:

Insulin is the primary anabolic hormone synthesized in the beta cells of pancreatic islets, and is important for the regulation of glucose and free fatty acids levels in the blood. It is a polypeptide hormone of the gene family of IGF-I, IGF-II and relaxin[57].

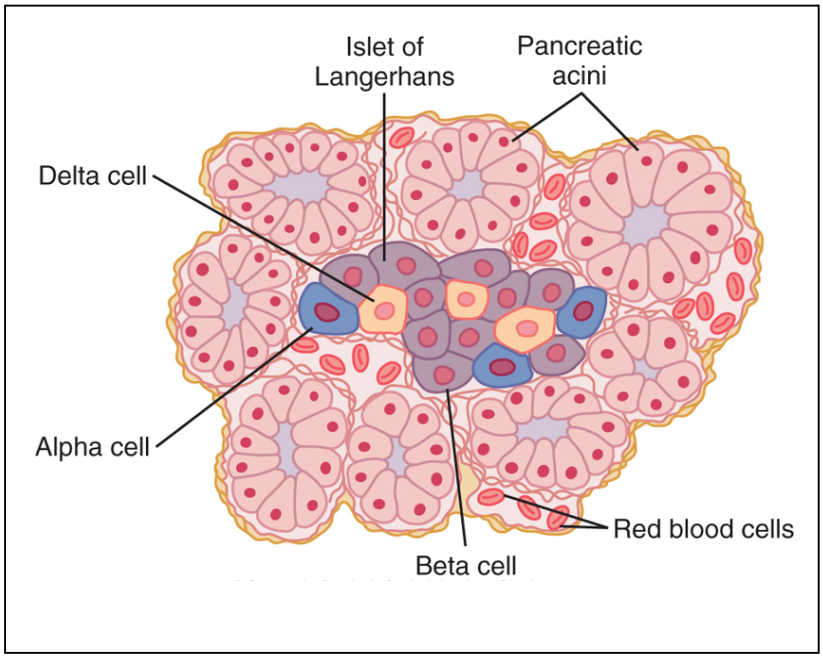
HISTORICAL ASPECTS:[55]

On 14th April 1921, Frederick.G.Banting, an orthopaedic surgeon along with his medical student Charles.H.Best started his work on isolation of antidiabetic hormone from the pancreas. He used his former professor JJR Macleod's laboratory for research work. In 1922, Banting and Best successfully extracted the hormone and injected it into the pancreas removed dogs. Then the dogs immediately recovered from their symptoms.

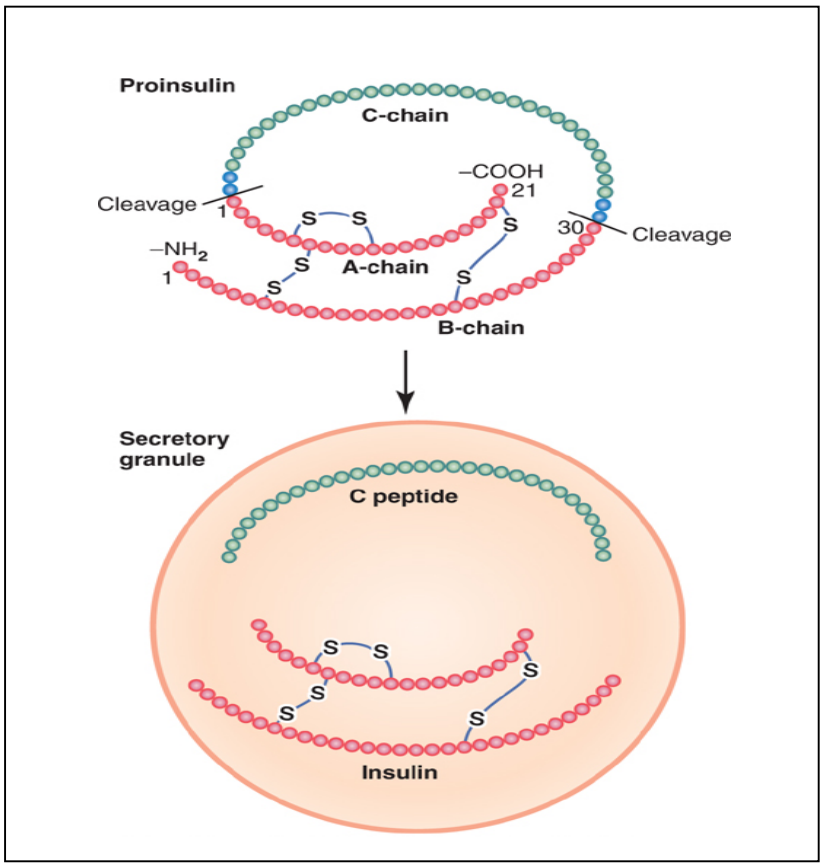
In 1923, for their wonderful job on discovery of insulin, Banting and Macleod were awarded with "NOBEL PRIZE". Banting provided a part of prize money to the Best. Likewise Macleod shared with JB Collip.

Professor Macleod and JB Collip along with his co-workers have successfully purified the insulin and first time injected it into a 14 years old boy Leonard Thompson. Daily injections reduced his blood sugar level and urine sugar. Ketone bodies also disappeared from his urine.

ISLET OF LANGERHANS



STRUCTURE OF INSULIN AND PROINSULIN



In 1934, Svedberg determined the molecular weight of insulin. In the middle of 1950s, Frederick sanger described about the molecular structure of insulin and got "NOBEL PRIZE" for his work on 1958.

STRUCTURE [58]

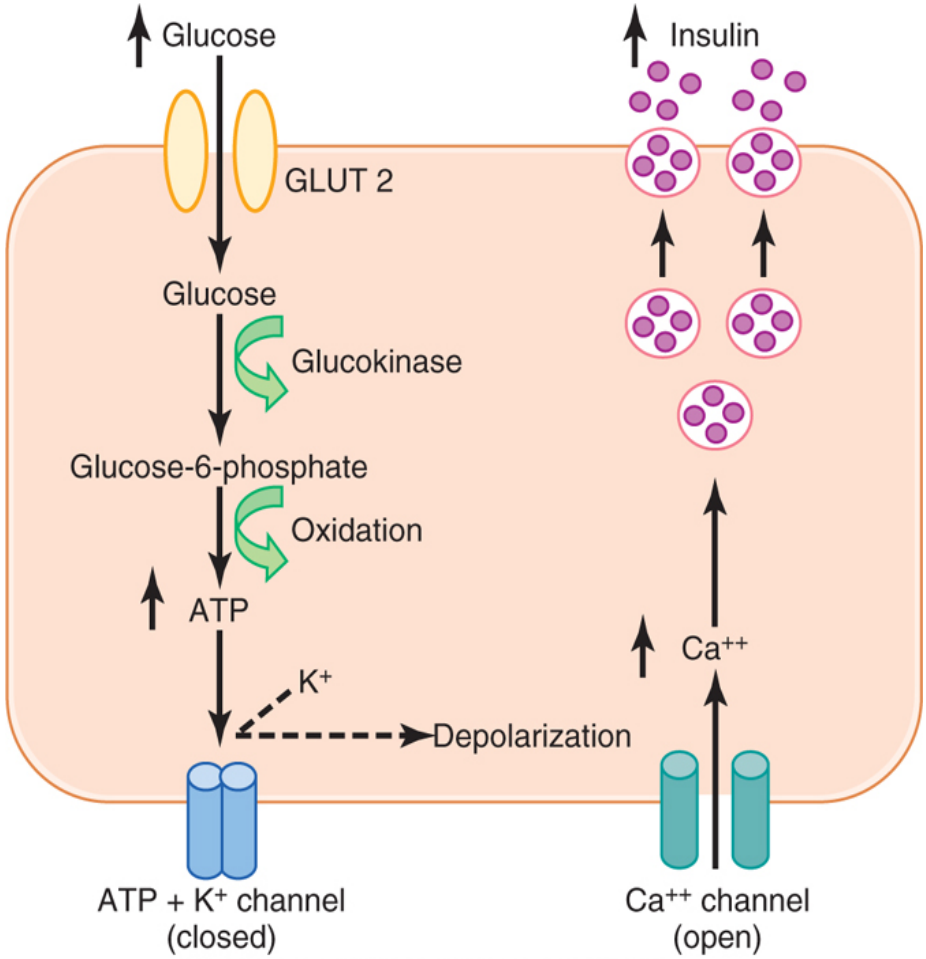
Insulin is a 51 aminoacids containing polypeptide hormone made up of two peptide chains namely α and β, α chain with 21 aminoacids and β chain with 30 aminoacids. These two peptide chains joined by two disulphide bonds and one more disulphide bond is present within the α chain itself. Molecular weight is about 5808 Da.

BIOSYNTHESIS [24,57,59]

Insulin gene located on the short arm of chromosome 11 and it codes the precursor hormone called Preproinsulin which is a 108 aminoacids containing polypeptide hormone synthesized in the polyribosomes.

In the endoplasmic reticulum,23 amino acid containing N terminal signal peptide is removed from Preproinsulin by the action of microsomal enzymes and Proinsulin is formed. This proinsulin constitutes A and B polypeptide chains of insulin and

MECHANISM OF INSULIN RELEASE



'C'peptide. 'C' peptide otherwise called as connecting peptide which facilitates the folding of proinsulin.

In the Golgi apparatus, membrane bound secretory granules are formed by the packaging of proinsulin. The secretory granules also contain propertein convertase 1/3 and 2 enzyme which cleaves the 'C'peptide from proinsulin molecule and forms the mature active hormone called "INSULIN".

Zinc combined with insulin forms zinc-insulin crystals which occupies the dense central core of the granules where as clear zone between the dense core and granular membrane is occupied by the 'C'peptide.

MECHANISM OF SECRETION [57,59]

The major stimulus for the insulin secretion is blood glucose. Glucose enters into the β cells through GLUT-2 transporters present on the cell membrane by facilitated diffusion. Glucose is converted into glucose 6 phosphate by the enzyme glucokinase, and is considered as the first rate limiting step in the insulin secretion process.

Glucokinase acts as glucose sensor and determines the rate of insulin secretion. If blood glucose is more, more glucose goes inside the beta cells, rate of glycolysis and ATP production

increases and which inturn increases rate of insulin secretion. If blood glucose is less, less glucose goes inside the beta cells, rate of glycolysis and ATP production decreases and which in turn decreases rate of insulin secretion.

Oxidation of glucose in the glycolytic pathway finally produces ATP molecules. K_{ATP} channels are closed by the elevated levels of ATP. This will lead to decrease in K^+ efflux and produce depolarisation inside the cell. SUR and KIR 6 & 2 are the components of K_{ATP} channels, and these combine to form the large octameric channel for the passage of K^+ ions.

Depolarisation causes opening up of voltage gated Ca^{2+} channels and increases intracellular Ca^{2+} level. Increased Ca^{2+} causes microtubule mediated exocytosis of granules and release their contents into the blood. 90-97% of released contents are insulin with equal quatities of 'C'peptide. The remaining released contents are proinsulin. About 50% 0f secreted insulin undergo first pass metabolism in the liver before reaching the peripheral circulation.

'C' peptide does not have specific biological action, and the measurement of 'C' peptide level by radioimmunoassay provides an index for internally secreted insulin level.

REGULATION OF INSULIN SECRETION	
STIMULATION	**INHIBITION**
NUTRIENTS: • **Glucose(primary stimulus)** • **Aminoacids** • **Fatty acids**	
HORMONES: • **Glucagon** • **GI peptides-GIP,GLP-I, Gastrin, CCK**	• **Somatostatin**
ANS : • **Parasympathetic stimulation(M_4R)** • **Sympathetic stimulation (β_2R)**	• **Sympathetic stimulation(αR)**

RATE OF INSULIN SECRETION [56,60]

Normal insulin release occurs in a pulsatile manner. It is characterised by rapid oscillations for each 8-15 minutes with superimposition of slower oscillations in every 80-150 minutes. Basal secretion rate in human is about 0.5-1 U/hr or 40 U/day because of burst off secretion after each meal.

METABOLISM OF INSULIN[59]

Insulin circulates in free form and its half life is about 5minutes. It is metabolised by the enzyme insulinase present in the kidney and liver.

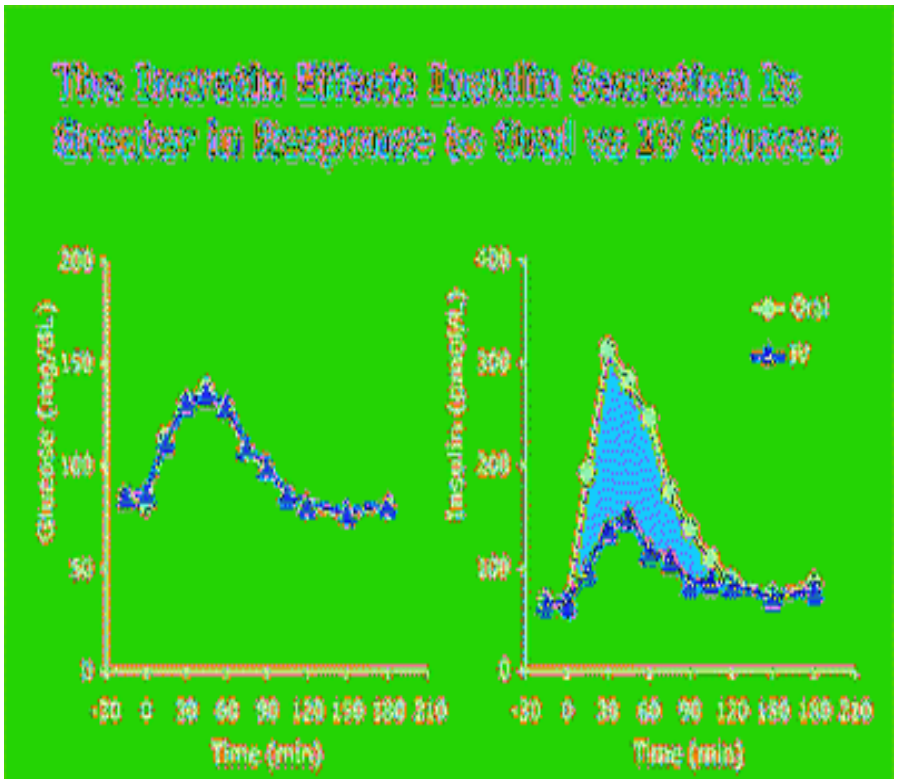
FACTORS REGULATING INSULIN SECRETION [56,60]

Carbohydrate nutrients:

Glucose is the essential food substance involved in the regulation of insulin secretion. Insulin secretion in response to oral glucose is more than the intravenously administered glucose. This is because of incretin hormones.

Incretins are the hormones secreted by the mucosa of small intestine in response to orally ingested carbohydrates and they are GLP-I and GIP . Incretins primes the beta cells to increase their response to orally ingested glucose. This phenomenon is called as “INCRETIN EFFECT”.

INCRETIN EFFECT



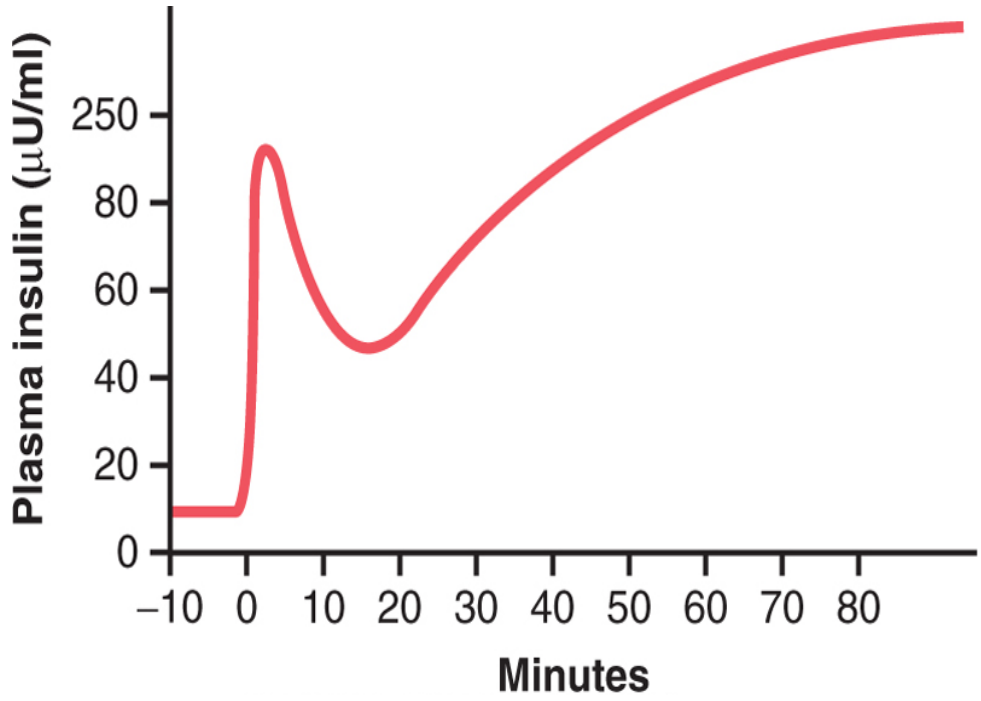
Intravenous infusion of glucose at a fixed rate causes biphasic insulin secretion .

Phase I: Rapid phase, peak attained within 1-2 minutes followed by abrupt fall and reaches the basal level in the next 5minutes. This is because of release of preformed insulin from the secretory granules. Lack of 1^{st} phase insulin secretion with postprandial hyperglycemia is the early feature of type II diabetes.

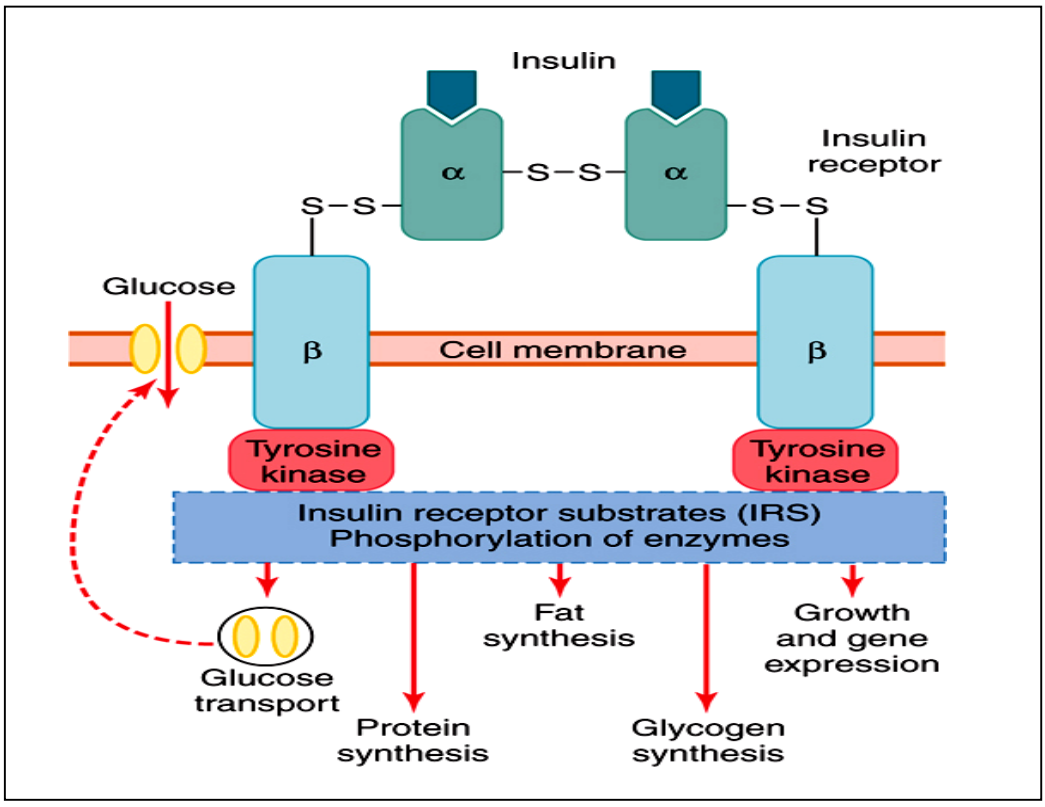
Phase II: Insulin secretion increases again but in a slow manner and attains the second peak. This phase depends on rise in blood glucose level. Decreasing 2^{nd} phase due to deterioration of β cell function results in marked hyperglycemia in diabetics.

This biphasic response is due to acute stimulating effect of glucose on insulin release. But prolonged exposure to higher glucose level decreases the insulin synthesis and secretion by decreasing the expression of insulin gene in the beta cells (glucotoxicity).

PHASES OF INSULIN SECRETION



MECHANISM OF ACTION OF INSULIN



Non carbohydrate nutrients:

Essential aminoacids like arginine, leucine and lysine increases the insulin secretion . Lipids and their metabolites have minimal effects on insulin secretion. Ketone bodies increases the insulin secretion during starvation.

Hormonal factors:

Hormones increasing the insulin secretion include GI hormones like GIP, -GLP-I, CCK and glucagon, glucocorticoids, sex steroids, prolactin , placental lactogen.

Neural factors:

Vagus nerve stimulates the insulin secretion via M_4 receptor and sympathetic fibres inhibit insulin secretion via α_1 receptor, and stimulates the insulin secretion via β receptor. Stimulation of ventromedian nucleus of hypothalamus decreases the insulin release.

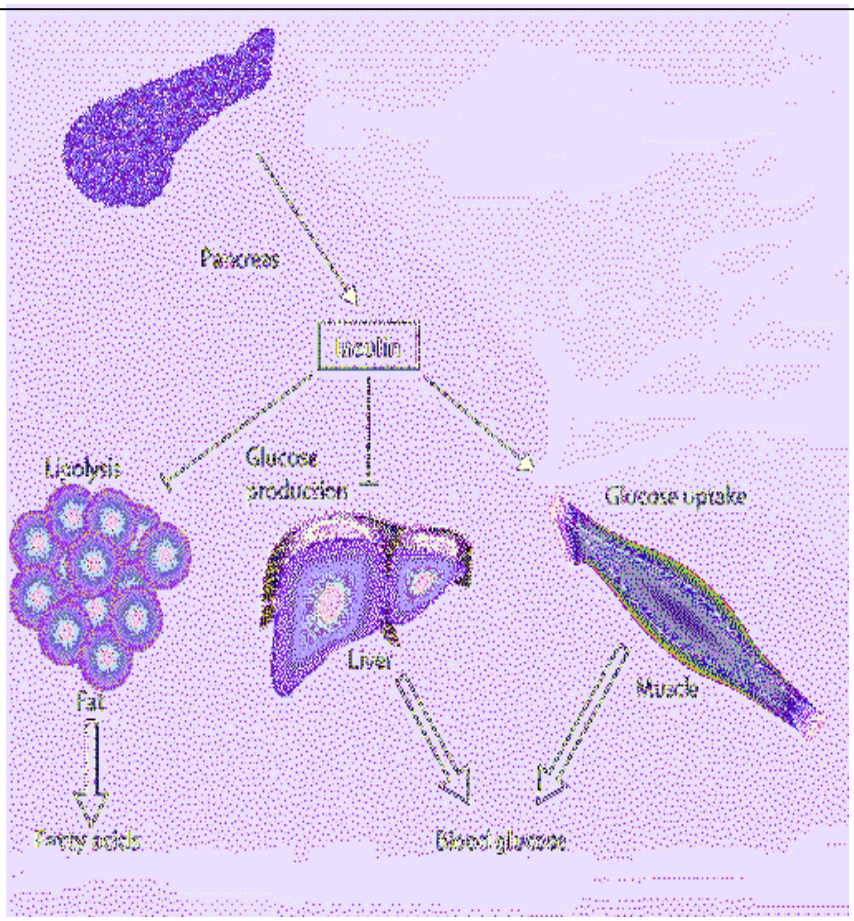
MECHANISM OF ACTION[57]

Insulin elicits its action by binding with insulin receptor located on the cell membrane of target tissues.

Steps involved in mechanism of action:

Insulin diffuses across the capillary wall and reaches the target tissues where it binds with α subunit of insulin receptor.

ACTIONS OF INSULIN



Autophosphorylation of β subunit occurs by the activation of tyrosine kinase enzyme. Phosphotyrosine residues of β chain causes recruitment of adapter proteins like IRS, Shc and APS protein. Finally the effects are expression of genes within the nucleus, synthesis of proteins like glucokinase, phosphofructokinase, deactivation and activation of enzymes involved in glucose and fatty acid metabolism and translocation of GLUT to the cell membrane.

PHYSIOLOGICAL ACTIONS OF INSULIN [28,57,59]

ACTIONS ON METABOLISM:

Insulin is the major endocrine hormone involved in the metabolism of carbohydrate, lipids and proteins.

ON CARBOHYDRATE METABOLISM:

In a normal healthy person, from the orally ingested glucose about 50% is used for giving energy to the body tissues by glycolysis. About 40% is converted and stored as fat and 10% of glucose is stored as glycogen. This type of balance between utilisation and storage of energy is mainly because of insulin hormone .

Insulin exerts its action mainly by acting on effector organs like skeletal muscle, adipose tissue and liver. Net effect of insulin on carbohydrate metabolism is to decrease the blood glucose level, and is considered as the only effective **"ANTIDIABETOGENIC HORMONE"** in the body.

Insulin decreases the blood glucose level by increasing the glucose uptake in the target tissues. This is achieved by translocation of GLUT transporters to the cell membrane. Insulin dependent glucose uptake occur in the muscle tissues like skeletal muscle, cardiac muscle, smooth muscle and adipose tissues, WBCs, Mammary glands. Insulin independent glucose uptake occurs in the tissues like nervous tissues, RBCs, Retina, Blood vessels and intestinal mucosa. In liver, glucose uptake is increased indirectly by increasing the utilisation of glucose not by the translocation of GLUT proteins.

In liver:

Insulin facilitates the glucose uptake by increasing the glucokinase action. Glucokinase increases the glucose utilisation by mediating the conversion of glucose into glucose 6 phosphate which inturn increases the glucose uptake via GLUT-2 transporter. Insulin induces glycolysis by enhancing the activity of

phospofructokinase and pyruvate kinase enzymes. It stimulates glycogen synthesis from glucose by inducing glycogen synthase enzyme complex activity. Then this glycogen is stored in the liver. By inhibiting the activity of glycogen phosphorylase and glucose 6 phosphatase enzymes it inhibits glycogenolysis. It also inhibits gluconeogenesis by preventing the entry of gluconeogenic aminoacids and by inhibiting the enzymes pyruvate kinase, phospoenol pyruvate carboxy kinase and fructose 1,6 bisphosphatase.

In adipose tissue:

Insulin increases the glucose uptake by promoting the translocation of GLUT-4 to the cellmembrane. Then the entered glucose is converted into α-glycerophosphate which is used in the esterification of fattyacids. By this process esterified fatty acids is stored as triglycerides.

In skeletal muscle:

Insulin increases the glucose uptake by promoting the translocation of GLUT-4 to the cellmembrane. Insulin increases glucose entry by 10-20 folds for few hours after meals. During exercise, muscle membrane permeability to glucose increases even in the absence of glucose. Because of this, the diabetic

patients are advised for regular exercise. Insulin facilitates the muscle glycolysis by stimulating the pyruvate dehydrogenase activity. It also promotes storage of glucose as glycogen in the muscle tissues.

ON FAT METABOLISM:

Insulin facilitates the storage of fat and reduces the fatty acid mobilisation and oxidation by following mechanisms.

In adipose tissue:

Insulin inhibits lipolysis by inhibiting the enzyme hormone sensitive lipase. By preventing lipolysis and further release of free fatty acids into the circulation insulin reduces the formation of ketoacids in the liver. So insulin is considered as major **"ANTIKETOGENIC HORMONE"** in the body.

Insulin induces the lipogenesis by activating the enzyme Lipoprotein lipase which is present in the vascular endothelium. Triglycerides and VLDL cholesterol cannot enter the adipose tissue directly. So they are converted into free fatty acids by the lipoprotein lipase , then these free fatty acids enter into the adipose tissue where they are modified and stored as triglycerides.

In Liver:

Insulin facilitates the synthesis of fatty acids from glucose by activating the enzyme acetylcoA carboxylase which mediates the conversion of acetylcoA to malonylcoA formation. This malonylcoA is used for the synthesis of fatty acids. It also stimulates the fatty acid synthase complex. In the HMP shunt, by promoting the action of glucose 6 phosphate dehydrogenase it increases the production of triphosphopyridines, which is needed for the fatty acid synthesis.

Insulin facilitates the cholesterol synthesis from acetylcoA by stimulating the enzyme HMGcoA reductase. Insulin is also helpful in the usage of VLDL and LDL proteins. So in diabetic patients in the absence of insulin action these VLDL and LDL protein level in blood may increase and they are involved in the pathogenesis of atherosclerosis.

ON PROTEIN METABOLISM:

Insulin promotes the synthesis of proteins in the muscle and liver. It promotes aminoacids entry into the muscle tissues. By stimulating the gene transcription and translation of mRNA, it stimulates protein synthesis on ribosomes. By reducing the lysosomal enzyme activity, insulin inhibits proteolysis. In liver,

insulin retains the aminoacids for protein synthesis by decreasing the gluconeogenesis.

Insulin deficiency in diabetics showed drastic reduction in the synthetic rate of albumin associated with significant increase in the level of plasma fibrinogen [2].

GLUCAGON[57]

29 aminoacids containing protein hormone secreted by the α cells of pancreatic islets. It is the **"PRIMARY COUNTER REGULATORY HORMONE"** responsible for increasing the blood glucose concentration. It increases the blood glucose level by increasing the gluconeogenesis and glycogenolysis in the liver. It also decreases glycolysis and lipogenesis from glucose in the liver.

PATHOPHYSIOLOGY OF TYPE II DIABETES MELLITUS

Pathophysiology of type II diabetes is complex and is in association with the involvement of environmental factors and genetic factors[56].

Genetic association of type II diabetes:

Type II diabetes has strong genetic susceptibility factor. Risk of getting diabetes is about 40% if both parents having type II diabetes. The chance of getting type II diabetes in identical twins is around 70-90%.

It is a polygenic form of disease with involvement of more than 20 genes. Some of the examples for genetic polymorphism are mutations present in the genes encoding the proteins like PPAR-γ, K^{+}_{ATP} channel, Zinc transporter, insulin receptor substrate protein and calcium dependent cysteine protease (calpain-10) [1].

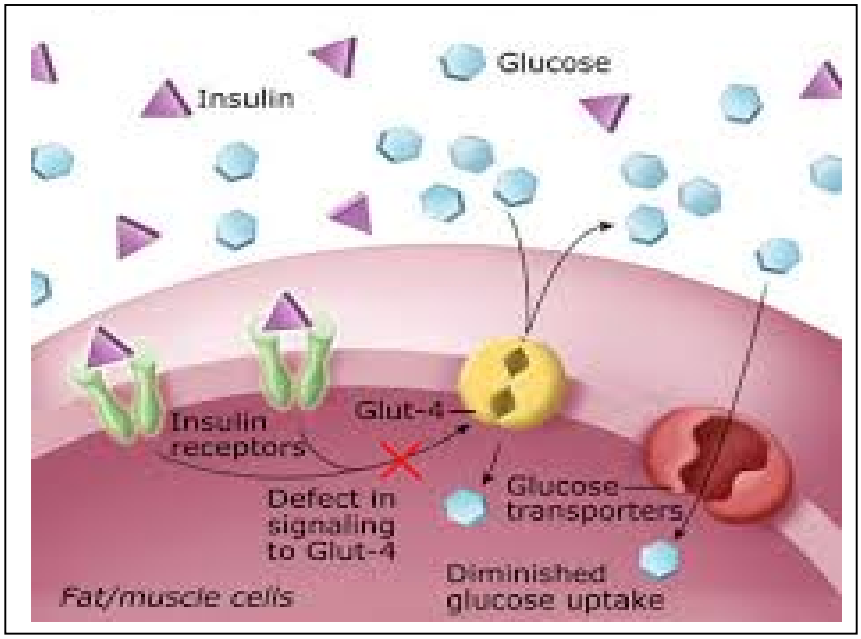
THE THREE CLASSICAL ABNORMALITIES OF TYPE II DIABETES INCLUDE,

- Insulin resistance
- Impaired insulin secretion by the β cells
- Increased glucose production by the liver

INSULIN RESISTANCE[55]

It is defined as decreased sensitivity of target tissues like skeletal muscle and adipose tissue to the insulin which is the predominant abnormality in type II diabetes mellitus[1].

TYPE 2 DIABETES: INSULIN RESISTANCE



Historical aspects of Insulin Resistance:

Gerald 'M' reaven was the person who first described about metabolic syndrome and he said that obesity and reduced physical activity each one is responsible for nearly 25% of insulin resistance and another 50% is by genetic factors.

In 1936, Himsworth was the first person who described that hyperglycemia in some diabetic patients is because of reduced insulin sensitivity in the target tissues other than insulin deficiency.

Genetic Factors include mutation of genes encoding ,

Insulin receptor substrate 1 & 2

Phosphatidyl inositol 3 kinase

GLUT transporter proteins

Liver glucokinase promoter gene

Environmental Factors:

It is considered as the major contributor of insulin resistance. It includes obesity, reduced physical activity and nutritional factors.

Obesity:

Central obesity is the most powerful risk factor of diabetes. Not only the increase in quantity of adipose tissue and also the adipose tissue dysfunction is responsible for Insulin resistance. Central adipocyte is more lipolytic than the adipocytes of peripheral tissues and releases more amount of non esterified fatty acids into circulation. These NEFA gets accumulated in the liver and skeletal muscle and reduces the insulin sensitivity by causing phosphorylation of serine residue instead of tyrosine residue at insulin receptor substrate proteins.

Central adiposity leads to increased release of adipokines like resistin, retinol binding protein-4 and decreased release of adiponectin and leptin into circulation. Resistin is the hormone responsible for insulin resistance. Normally leptin and adiponectin increases the insulin sensitivity by stimulating the AMP activated protein kinase enzyme action. This enzyme in turn promotes fattyacid oxidation in the liver and skeletal muscle. Increased resistin and decreased adiponectin together decreases the insulin sensitivity in target tissues[31].

Reduced physical activity:

Urbanisation of the country has lead to reduced physical activity. It is the another factor responsible for more prevalence of diabetes among urban people than in the rural one.

Nutritional factors:

Nutritional factors like excess calorie intake in the form of high carbohydrate diets like refined flour, pasta, raw rice, aerated soft drinks, sweet, sugar leads to type 2 diabetes. Also the intake of high saturated fat diets like coconut oil, vanaspathi, omega6 fatty acid rich foods like corn and decreased intake of vegetables and fruits. Dietary deficiency of chromium, zinc, selenium and also vitamin D deficiency can lead to diabetes.

INSULIN RESISTANCE ASSOCIATED METABOLIC ABNORMALITIES :

Decreased glucose utilisation by the target organs like adipose tissues and skeletal muscles is due to reduced insulin sensitivity . Increased gluconeogenesis in the liver leading to entry of more glucose into the blood which finally results in hyperglycemia. Increased hepatic gluconeogenesis is responsible for increase in fasting plasma glucose concentration whereas

decreased peripheral utilisation of glucose is responsible for postprandial hyperglycemia in diabetes mellitus.

In skeletal muscles and liver, the glycogen storage is also affected. In adipose tissue, increased lipolysis leading to release of more free fatty acids into circulation results in increased content of VLDL proteins and triglycerides in the blood[1].

IMPAIREMENT OF INSULIN SECRETION [55]

It occurs due to progressive β cell dysfunction with decrease in β cell mass.

Phase I: Increase in the β cell mass to overcome the insulin resistance leads to hyperinsulinemia.

Phase II: (stage of prediabetes) It is the early stage of β cell dysfunction. In this phase glucose induced β cell response is affected. But response to other stimulants remains normal.

Phase III: In this phase there is a gross reduction in the response of β cells to glucose stimulus along with decrease in response to other secretogogues.

Phase IV: There is absence of β cell proliferation in this phase. This is because of chronic hyperglycemia which results in 40-50% reduction in the β cell mass.

COMPLICATIONS OF TYPE II DIABETES[1]

ACUTE COMPLICATIONS:

- Diabetic ketoacidosis
- Hyperglycaemic hyperosmolar state.

CHRONIC COMPLICATIONS:

- **MICROVASCULAR DISEASES:**
  - Retinopathy
  - Neuropathy – sensory, motor, autonomic.
  - Nephropathy

- **MACROVASCULAR DISEASES:**
  - Coronary artery disease (CAD)
  - Peripheral arterial disease (PAD)
  - Cerebrovascular disease.

Chronic complications like microvascular and macrovascular complications are responsible for most of the diabetes associated morbidity and mortality. Regarding macrovascular diseases, the occurrence of coronary artery diseases and associated mortality risk is 2-4 times higher in type II diabetic patients. Multiple factors are involved in the pathogenesis of macrovascular diseases and these are chronic hyperglycemia, hypercholesterolemia, and Systemic hypertension. But the central mediator of this pathogenesis is the glucotoxicity.

THEORIES OF COMPLICATIONS: [1,31]

1st theory said that advanced glycation end products (AGE) like glyoxal, methyl glyoxal and 3-deoxy glucosone are formed by raised intracellular glucose. Then these AGEs bind with receptor called RAGE (Receptor of advanced glycation end products) present on the macrophages, endothelium and vascular smooth muscle by non enzymatic glycosylation of both intra and extracellular proteins.

Advanced glycation end products- Receptor of Advanced glycation end products complex in the blood vessels causes production of oxygen free radicals in the endothelium, which enhances the procoagulant action on endothelial membrane of the blood vessel.

AGEs can also cause cross linking of extracellular matrix proteins. Cross linking of type I collagen in large blood vessels leads to decreased elasticity and endothelial injury. Cross linking increases protein deposition leading to accumulation of more and more LDL proteins in the tunica intima of large vessel walls accelerating the atherogenesis.

The 2nd theory proposed that, in case of excess intracellular glucose, some amount of glucose is metabolised into sorbitol by the enzyme aldose reductase . Increased sorbitol induces oxygen free radicals formation which inturn causes cellular dysfunction.

The 3rd theory said that, increase in production of diacyl glycerol causes activation of Protein kinase – C. The activated Protein kinase – C induces the production of plasminogen activator inhibitor-1 which inhibits fibrinolysis. It will also promote the formation of vascular endothelial growth factor-1 leading to neovascularisation. Protein kinase –c activation is

associated with decreased expression of eNOS leading to decreased nitric oxide level which also accelerates endothelial injury.

GLYCOSYLATED HEMOGLOBIN[55]

In present days, HbA1c is considered as s best tool for assessing the long term glycemic control in diabetic patients.

HISTORICAL REVIEW:

In 1955, Kunkel and his co workers observed that the haemoglobin consists of fast and slow moving components of electrophoresis.

Rahbar found that, the fast components of haemoglobin was elevated in diabetics with poor metabolic control.

Bunn and Nathan described about the clinical correlation of HbA1c. Goldstein studied the kinetics of HbA1c.

PROCESS OF GLYCOSYLATION:

It occurs by binding of glucose with β chain of HbA at its amino terminal valine. This will alter the pattern of movement in cation exchange chromatography and is the major glycosylated haemoglobin called HbA1c . Normal value is 4 - 6%.

The life span of RBC is about 120 days, during which haemoglobin gets attached with glucose molecule to from glycosylated haemoglobin. In diabetes mellitus patients with poor glycemic control, the quantity of glycosylated haemoglobin is in increased amount when compared to patients with good glycemic control[61].

IMPORTANCE OF GLYCEMIC CONTROL:

In diabetes mellitus, management of the disease is mainly focussed on prevention of chronic complications and to interfere with the progression of disease.

The United kingdom prospective diabetes (UKPDS) study established the relationship between good glycemic control and prevention of complications in type II diabetes mellitus.

The American diabetes association recommended HbA1c level of <7% as a goal for good glycemic control in diabetic patients[1].

DIABETES - PROTHROMBOTIC STATE[62]

Endothelial dysfunction:

Vascular endothelium acts as a physical barrier between blood and vessel wall. It is also essential for maintaining the balance between procoagulant and anticoagulant system of blood. Smooth surface of endothelium prevents the platelet adhesion, aggregation and blood coagulation, whereas induces the fibrinolysis.

In diabetes mellitus, irreversible glycation of subendothelial collagen and structural proteins forms the AGEs. These glycation end products produce alterations in the basement membrane structure leading to endothelial injury and increased vascular permeability.

vWF is a multimeric protein synthesised and stored within the weibel palade bodies present in the endothelium of blood vessels. During endothelial injury, vWF released into circulation leading to hypercoagulablity of the plasma. Positive correlation is observed between increased vWF and vascular complications of type II diabetes mellitus.

Changes in platelets:

Glycation of platelet membrane proteins is associated with change in the shape of platelets, reduced membrane fluidity, increased platelet activation, increase in number of GP Ib and GPIIb-IIIa receptor complexes finally leading to platelet plug formation.

Changes in clotting factors:

Fibrinogen is an acute phase protein which gets elevated in type II diabetes mellitus patients and is considered as an independent risk factor for cardiovascular diseases. Glycation of fibrinogen molecule leading to formation of dense fibrin clot with fine fibres which are less sensitive to fibrinolysis. Glycated fibrin binds with t-PA and plasminogen in lesser amount, but binds to α2 antiplasmin more avidly leading to production of less plasmin. This in turn is responsible for impaired fibrinolysis[63].

Diabetes associated dyslipidemia increases clotting factor VII (FVII) in the blood. Normally, part of FVII is bound to triglyceride - rich VLDL. Because of increased VLDL content in diabetes, FVII in the plasma increases along with lengthening of half life of FVII.

Factor VIII/ vWF complex is also increased in type II diabetes , because of endothelial injury and inflammation associated increase in vWF. Half life of vWF bound F VIII is increased from 37 minutes to 24.5 hrs.

Changes in fibrinolysis[60]

Fibrinolysis is a naturally existing mechanism, which prevents the spread of blood clot away from the site of injury by causing dissolution of blood clot. Plasmin or fibrinolysin is the active substance involved in fibrinolysis [25,28].

Plasmin is a serine protease enzyme present in the plasma, which cleaves lysine residues and arginine residues at all the sites in fibrin and fibrinogen. Then fibrin molecules are lysed and broken down to form small soluble substances called fibrin degradation products.

Plasminogen Activators :

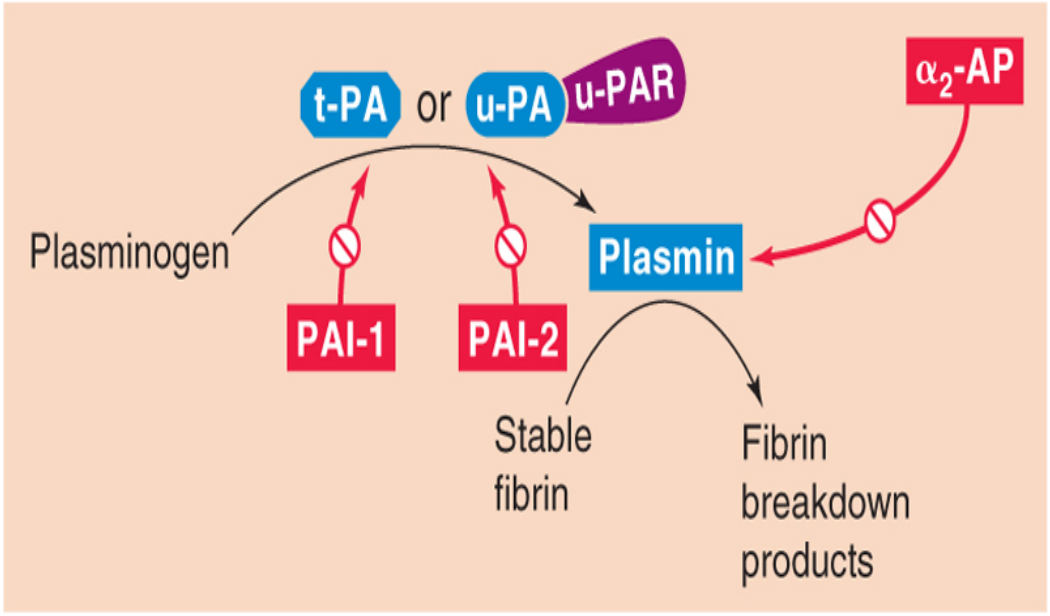
These are serine protease enzymes present in the plasma. They cleave single covalent disulfide bond from the inactive precursor plasminogen and converts it into active substance called plasmin. The two different plasminogen activators are t-PA and u-PA. The t-PA is secreted by endothelial cells whereas u-PA is secreted by endothelial cells, monocyte and fibroblasts. Fibrin

deposited at the site of vessel injury and circulating epinephrine act as stimulants for the release of t-PA. Single chain t-PA and u-PA initiate fibrinolysis through their synergistic action.

Single chain u-PA acts on fibrin bound plasminogen and converts it into plasmin. Plasmin removes the peptide bonds present at carboxy terminal of fibrin and exposes other carboxy terminal lysine residues, by which plasmin cleaves subsequent peptide bonds and forms fibrin degradation products.

Fibrin Degradation Products:

Plasmin cleaves the peptide bonds present in the fibrinogen or fibrin in a sequence and forms large degradation products called X and Y and smaller degradation products called D and E. Large degradation products inhibit fibrin polymerisation whereas X,Y and D fragments inhibit binding of fibrinogen to GPIIb-IIIa receptors, hence prevent platelet aggregation.



Plasminogen activator inhibitors:

These substances play a major role in fibrinolysis regulation process. There are two types, PAI-1 and PAI-2. PAI-1 is secreted by the endothelial cells and also stored in the α-granules of platelets. PAI-1 inhibits both t-PA and u-PA. PAI-2 is mainly secreted by the placental macrophages and inactivates u-PA.

Increased PAI-1 in diabetes :

In type II diabetes, elevated levels of PAI-1 inhibits t-PA and u-PA leading to decreased production of plasmin and impaired fibrinolysis. PAI-1 levels show circadian changes with maximum levels in the morning and this is responsible for the increased incidence of acute myocardial infarction in the morning.

Hypercoagulability is associated with impaired fibrinolysis leading on to procoagulant state in diabetes. Glycation of fibrinogen results in alteration in the structure of formed clot with less susceptibility to clot lysis. This is because, thin fibrin fibers of modified clot bind less avidly to plasminogen and tPA whereas bind more avidly with antiplasmin[63].

STUDIES RELATED TO FIBRINOGEN AND DIABETES MELLITUS

A cross sectional study done among 1574 type II diabetes mellitus patients by Bruno G et al observed the high prevalence of hyperfibrinogenemia in diabetes and plasma fibrinogen level was significantly associated with HbA1c value [65].

In another cross sectional study done by Taj Muhammad Khan and his co-workers found, significantly high plasma fibrinogen level and increased plasma viscosity in diabetic patients. They also observed significantly increased plasma fibrinogen level in diabetics with complications [66].

Nizar and Elshazali described that plasma fibrinogen level was increased in type II diabetes mellitus patients than in controls, but it was in the higher limit of normal range (3.96±0.92).

The mean plasma fibrinogen level was slightly increased in diabetic patients with poor glycemic control than in patients with good glycemic control [67].

In the study of A.S. Bembde, plasma fibrinogen level was significantly increased in type II diabetic patients when compared to healthy subjects. Plasma fibrinogen level was also associated with other cardiovascular risk factors like systemic hypertension, obesity and smoking. Diabetic patients with poor glycemic control had higher mean plasma fibrinogen level. Plasma fibrinogen levels were positively correlated with glycosylated haemoglobin level[68].

In another cross sectional study conducted at Tribhuvan university teaching hospital by Dr Kafle et al found, significantly higher plasma fibrinogen level in type 2 diabetic patients. They also observed that plasma fibrinogen level was significantly correlated with glycosylated haemoglobin[69].

Antonio cereillo et al observed significantly increased plasma fibrinogen level in diabetics. Plasma fibrinogen in diabetics was positively correlated with thrombin activation parameters and suggested that fibrinogen might be a cardiovascular risk factor in diabetes mellitus patients [70].

Ying Zhao and co-workers found significantly reduced activated partial thromboplastin time and elevated plasma fibrinogen level in diabetic subjects. Significant changes in the above parameters were also observed in persons with impaired fasting glucose when compared to normoglycemic subjects[71].

In the study of Ritu Madan et al, significantly increased levels of PAI-1, plasma fibrinogen and vWF activity were observed in diabetic patients when compared to healthy control group. They said that diabetes is a hypercoagulable state which contributes to the cardiovascular complications of diabetes mellitus[72].

In the Framingham off spring study done by James J.Stec et al, increased plasma fibrinogen level correlated with conventional cardiovascular risk factors like diabetes mellitus, systemic hypertension , smoking and dyslipidemia[17].

According to Venkataramana.G et al, in type II diabetes mellitus, total proteins as well as fibrinogen was significantly increased whereas serum albumin was significantly decreased than in control group. They said that, this increased fibrinogen may also be due to increased plasma glucose in diabetes [73].

Rocco Barazonni and co-workers found that, on exogenous insulin infusion, plasma fibrinogen concentration was increased because of increase in both fractional and absolute synthetic rate by 41% and 43% respectively[74].

In a study done by P De Feo et al, insulin deficiency in diabetic patients was associated with 50% increase in fibrinogen synthesis and 29% decrease in albumin synthesis with p value of <0.03. They said that, increased plasma fibrinogen level was due to insulin deficiency associated metabolic stress on the liver[18].

In the study of Danuta Milosz et al, mean Plasma Fibrinogen level was significantly elevated in diabetics with severe coronary atherosclerosis with the p value of <0.05 [75].

Rodolfo Guardado-Mendoza et al said that, fibrinogen is the strong predictor of silent myocardial ischemia and it should be included in the cardiovascular risk profile of diabetic patients. They were also found a significant correlation between plasma fibrinogen and glycosylated haemoglobin[76].

MATERIALS & METHODS

MATERIALS AND METHODS

STUDY DESIGN:

This is a cross-sectional study.

STUDY PLACE:

The study was carried out in the department of Physiology, in association with department of Diabetology, Coimbatore Medical College Hospital, Coimbatore.

STUDY PERIOD:

The study was conducted from August 2013 to June 2014.

INCLUSION CRITERIA:

- Age and sex matched healthy individuals taken as control group in the study.
- Type II diabetic patients of both sexes in the age of 40-60 years.

EXCLUSION CRITERIA:

Patients with history of,

- Type I diabetes mellitus
- Hypertension
- Smoking
- Obesity
- Coagulation disorders
- Recent surgery
- Drug intake - Oral contraceptive pills
- Anticoagulants like Heparin, Warfarin.
- Renal diseases
- Liver diseases
- Pregnancy
- Postmenopausal state
- Infectious diseases
- Inflammatory conditions
- Hypercholesterolemia
- Vascular complications of Diabetes mellitus

STUDY SUBJECTS:

After fulfilling all the inclusion and exclusion criteria, **150** subjects were selected for the present study. The sample population was grouped into type II diabetes mellitus patients **(75)** and control group (**75).** Subjects in control group were healthy volunteers accompanying the patients.

Materials used for the study:

1. Proforma : To obtain detailed history, to record the vital parameters and to measure the anthropometric indices.
2. Portable weighing machine – to record the body weight in kilograms.
3. Stadiometer – to measure the standing height in centimeters.
4. Standardized mercury sphygmomanometer – to record the Blood Pressure.
5. Quantimate turbidimetry analyser – To measure plasma fibrinogen, glycosylated haemoglobin (HbA1c) .
6. Auto analyser – Random blood sugar

Methodology:

- After obtaining clearance from the institutional ethical committee, the subjects were selected and grouped. The subjects were explained about procedure in detail and informed consent was obtained.

I) History taking and clinical examination:

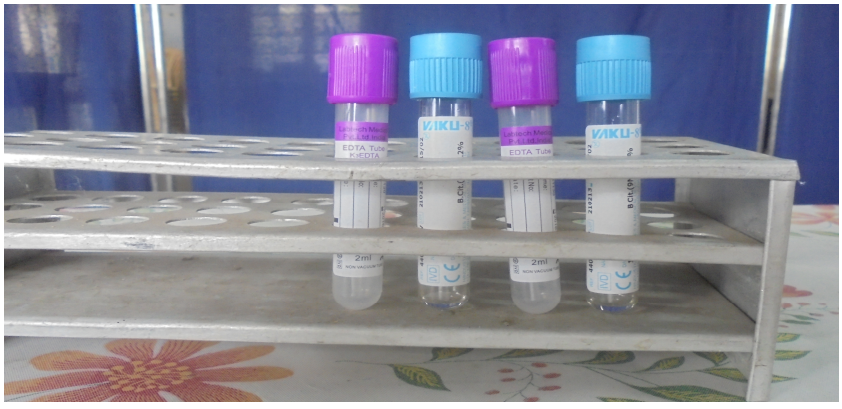
- Detailed history was elicited from the subjects to rule out signs and symptoms of hypertension, cardiovascular diseases, liver and kidney diseases and other causes influencing plasma fibrinogen level.
- A thorough clinical examination of all systems of body was done.

II) Measurement of anthropometric indices:

a) **Weight of subject:** The subjects were asked to stand erect with their arms relaxed at their side, with both feet close together. By using a portable standard weighing machine, weight in kilograms was recorded .

COLLECTION OF BLOOD SAMPLE

ANTICOAGULANT ADDED CONTAINERS



b) **Height of subject:** By using a stadiometer , height of subject in centimetres was measured by asking the subject to stand erect and the vertical height was measured.

c) **BMI of subject:** Body mass index was calculated by using quetelet's index.

BMI = Weight (Kg) / Height^2 (m)

III) Measurement of Blood Pressure:

First, the subjects were asked to sit quietly for 15 minutes in a quiet room with comfortable room temperature. Then blood pressure was recorded in all subjects by using a standard sphygmomanometer having a cuff size of 25 x 12.5 cms.

IV) Blood investigation:

Median cubital vein in the front of forearm was selected for venous blood collection. The skin over the vein was cleaned with spirit and cotton swab and allowed to dry. Then a disposable sterile needle fitted with 10 ml syringe was introduced in to the vein and 4ml of blood was collected and poured into separate containers having different anticoagulants.

TURBIDIMETRY

FIBRINOGEN KIT

3.2% sodium citrate anticoagulant added blood was used for measuring plasma fibrinogen and random blood sugar.

The anticoagulant EDTA (Ethelyne Diamine Tetra Acetic acid) mixed whole blood is used for measuring glycosylated haemoglobin (HbA1c).

METHODS OF MEASUREMENT:

1. **Turbidimetric immunoassay :**

   Plasma fibrinogen

   Glycosylated haemoglobin (HbA1c)

2. **Glucose oxidase-peroxidase method :**

   Random blood sugar

TURBIDIMETRIC IMMUNOASSAY

Measurement of Plasma fibrinogen

Reagents used:

R1 - activation buffer

R2 - anti fibrinogen antibody

Principle:

The method is based on aaglutination reaction. Reagent R1 and R2 are added to test sample. Fibrinogen present in the test sample react with anti fibrinogen antibody and forms insoluble complex with increasing turbidity. Increase in turbidity depends on concentration of fibrinogen in the test sample and it was measured at 340nm.

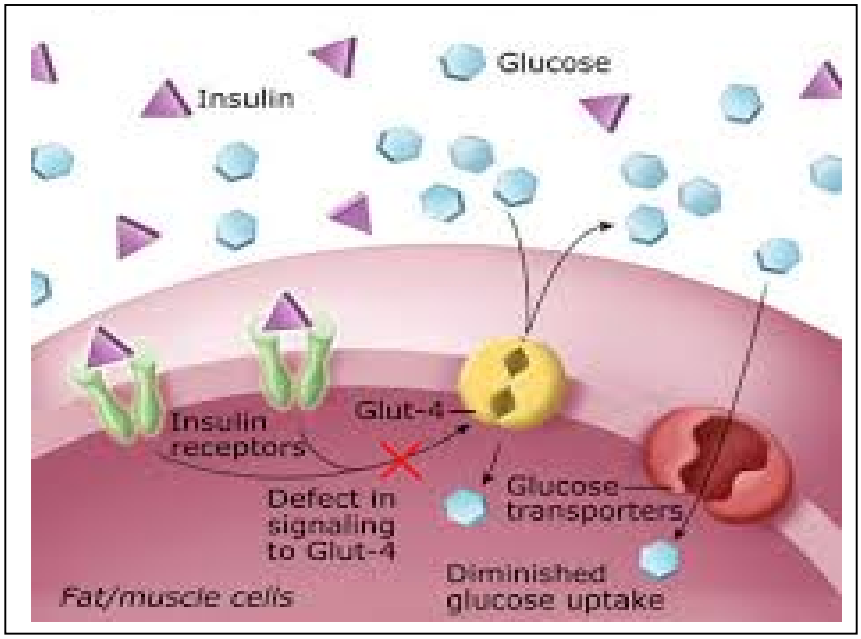
Specimen collection and preparation:

Freshly collected blood was added with 3.2% sodium citrate solution and centrifuged immediately for 5minutes at 3000rpm.then plasma was separated and transferred into a clean micropipette.

Test procedure:

Test specimen was diluted with normal saline in the ratio of 1:10. Then 0.5ml of activation buffer and 0.02ml of test sample were added and mixed thoroughly in the cuvette then the mixure was incubated at 37^{0} C for 5minutes. Immediately absorbance (A1) was read. In the next step, 0.1ml of anti fibrinogen antibody was added and absorbance (A2) was read at the end of 5minutes. ΔA (A2-A1) was calculated for test sample.

TYPE 2 DIABETES: INSULIN RESISTANCE



Then the test was carried out with different dilution of test sample such as, 1:20, 1:40, 1:60. ΔA of diluted test sample was interpolated on the calibration curve and fibrinogen concentration was measured.

Measurement of glycosylated haemoglobin (HbA1c)

Reagents used:

R1 - latex particles

R2 - mouse antiHbA1c antibody solution

R3 - Goat antimouse human IgG antibody solution

Quantia hemolysing reagent solution.

Principle:

Immunoassay method is based on agglutination reaction. After adding hemolysing solution, test sample is allowed to react with latex Reagent (R1). The amount of binding depends on relative concentration of HbA1c in the blood. Then the mixure is allowed to react with mouse antiHbA1c antibody reagent (R2), wherein R2 bind to latex bound HbA1c molecules. Goat antimouse human IgG antibody (R3) binds with HbA1c-R2

complex by agglutination reaction and it should be measured at 630nm. Change in turbidity of sample depends on concentration of HbA1c in the sample.

Test sample preparation:

Blood sample was thoroughly mixed for the uniform distribution of red blood cells. Then 0.5ml of hemolysing agent was added with 0.01ml of test sample and and which was mixed well and wait for 15 minutes for complete hemolysis. Hemolysed sample is called as lysate.

Test procedure:

0.4ml of latex particle (R1) was taken in a measuring couvette. Then 0.01ml of lysate was added with R1 and it was incubated at 37 ^{0}C for 2 minutes. After adding of 0.1ml of mouse antiHbA1c antibody reagent (R2), solution was thoroughly mixed and again incubated for 3 minutes. Then 0.02ml of Goat antimouse human IgG antibody reagent (R3) was added and solution was gently mixed.

The absorbance A1 was read at 10 seconds and absorbance A2 was read at the end of 2 minutes. ΔA (A2-A1) was calculated for test sample and calibration curve was plotted against %HbA1c and concentration of HbA1c for test sample was measured.

GLUCOSE OXIDASE-PEROXIDASE METHOD

(TRINDER'S METHOD)

Measurement of Random Blood Sugar

Glucose present in the test sample was oxidised to produce gluconic acid and hydrogen peroxide in the presence of glucose oxidase enzyme. Then the enzyme peroxidise promotes the reaction between 4-aminoantipyrine and phenol to yield quinoneimine dye complex. absorbance was read and which was correspond to the glucose concentration in the test sample.

After thorough clinical examination and blood investigations study population were divided into two groups and categorised as,

Group I - **75** Type II diabetes mellitus patients

Group I A - Type II diabetes mellitus patients with HbA1c $\leq$ 7% (good glycemic control)

Group I A1 - diabetes mellitus with duration of less than 5 years

Group I A2 - diabetes mellitus with duration of more than 5 years

Group I B - Type II diabetic patients with HbA1c>7% (poor glycemic control)

Group I B1 - diabetes mellitus with duration of less than 5 years

Group I B2 - diabetes mellitus with duration of more than 5 years

Group II - **75** age and sex matched healthy individuals as controls.

STATISTICAL ANALYSIS

STATISTICAL ANALYSIS

The information collected regarding all the selected cases were recorded in a Master Chart. Data analysis was done with the help of computer using **Epidemiological Information Package (EPI 2010)** developed by Centre for Disease Control, Atlanta.

Using this software, range, frequencies, percentages, means, standard deviations, chi square, and 'p' value were calculated. Unpaired 't' test was used to test the significance of difference between quantitative variables and Yate's and Fisher's chi square tests for qualitative variables. A 'p' value less than 0.05 is taken to denote significant relationship. Correlation coefficient was calculated using Excel software. A value greater than $\pm$ 0.5 is taken to indicate the existence of correlation between the variables.

RESULTS

RESULTS

Study subjects of both sexes were divided into two groups and categorized as:

Group I - **75** Type II diabetes mellitus patients

Group I A - **37** Type II diabetes mellitus patients with HbA1c $\leq$ 7% (good glycemic control)

Group I A1 - diabetes mellitus with duration of less than 5 years

Group I A2 - diabetes mellitus with duration of more than 5 years

Group I B - **38** Type II diabetic patients with HbA1c>7% (poor glycemic control)

Group I B1 - diabetes mellitus with duration of less than 5 years

Group I B2 - diabetes mellitus with duration of more than 5 years

Group II - **75** age and sex matched healthy individuals as controls.

TABLE 1:COMPARISION OF HEIGHT ,WEIGHT AND BMI BETWEEN GROUP I AND GROUP II

Group	Height		Weight		BMI	
	Mean	SD	Mean	SD	Mean	SD
Study group	157.1	6.2	61.7	6.1	24.9	1.5
Control Group	155.6	6.4	60.6	6.3	25.2	2.0
'p'	**0.1331 Not significant**		**0.3051 Not significant**		**0.2768 Not Significant**	

There is no statistical difference in Height, weight and BMI between study group and control group and the groups were comparable.

FIGURE 1:

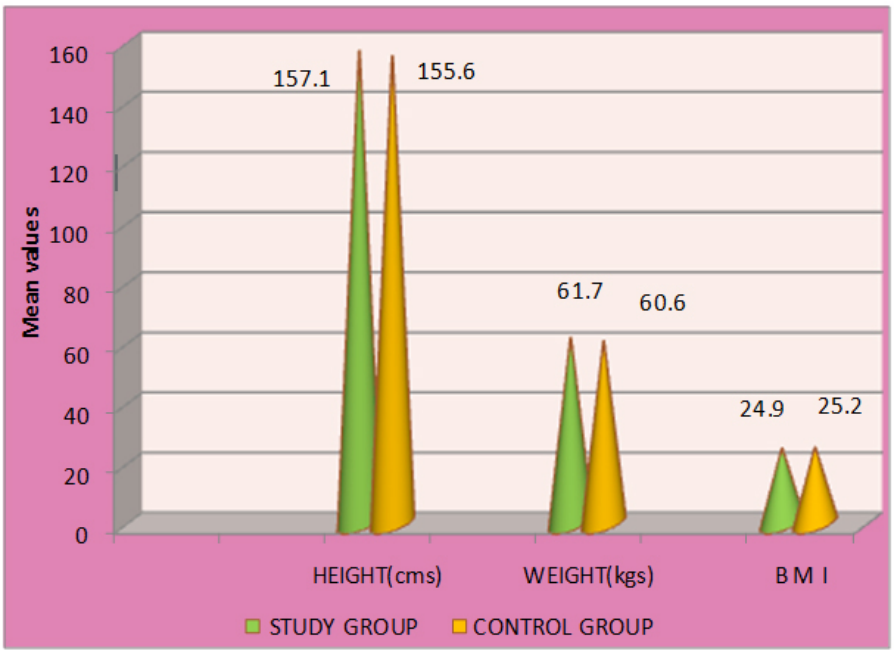


TABLE 2 : COMPARISION OF BLOOD PRESSURE BETWEEN GROUP I AND GROUP II

Group	Systolic BP		Diastolic BP	
	Mean	SD	Mean	SD
GROUP I	112.2	6.8	73.6	4.1
GROUP II	114.7	5.2	75.0	4.3
'p'	0.112 Not significant		0.0663 Not Significant	

There is no statistical difference in blood pressure between study group and control group and the groups were comparable.

FIGURE 2:

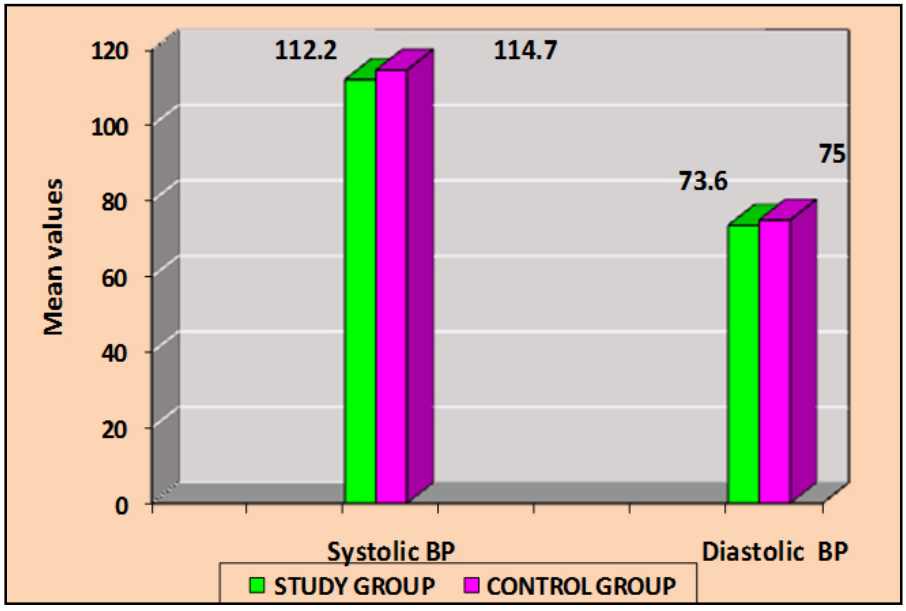


TABLE 3: COMPARISION OF PLASMA FIBRINOGEN LEVEL BETWEEN GROUP I AND GROUP II

GROUP	Plasma Fibrinagen (mgs / dl)	
	Mean	**SD**
GROUP I	276.6	87.9
GROUP II	160.3	49.7
'p'	**<0.0001 Significant**	

Statistically significant difference was present between study group and control group.

FIGURE 3:

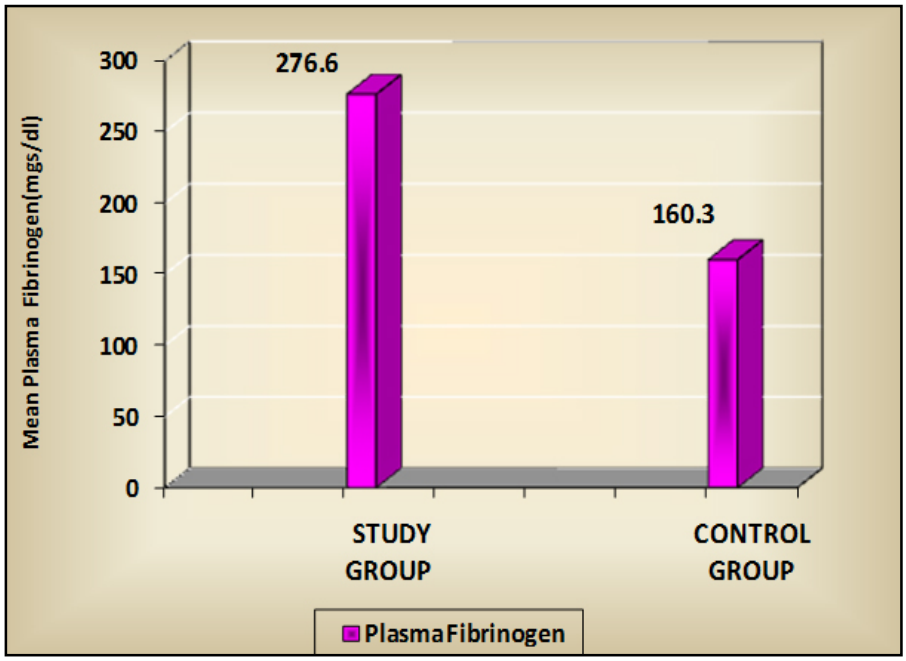


TABLE 4: COMPARISION OF PLASMA FIBRINOGEN LEVEL BETWEEN GROUP IA1 AND GROUP IA2

Group	Plasma Fibrinogen (mgs / dl)	
	Mean	**SD**
Group IA1 s	202.7	58.2
Group IA2	235.3	36.4
'p'	0.0641 Not significant	

Plasma fibrinogen level increased in patients with more than 5 years duration than in patients with less than 5 years duration.

FIGURE 4:

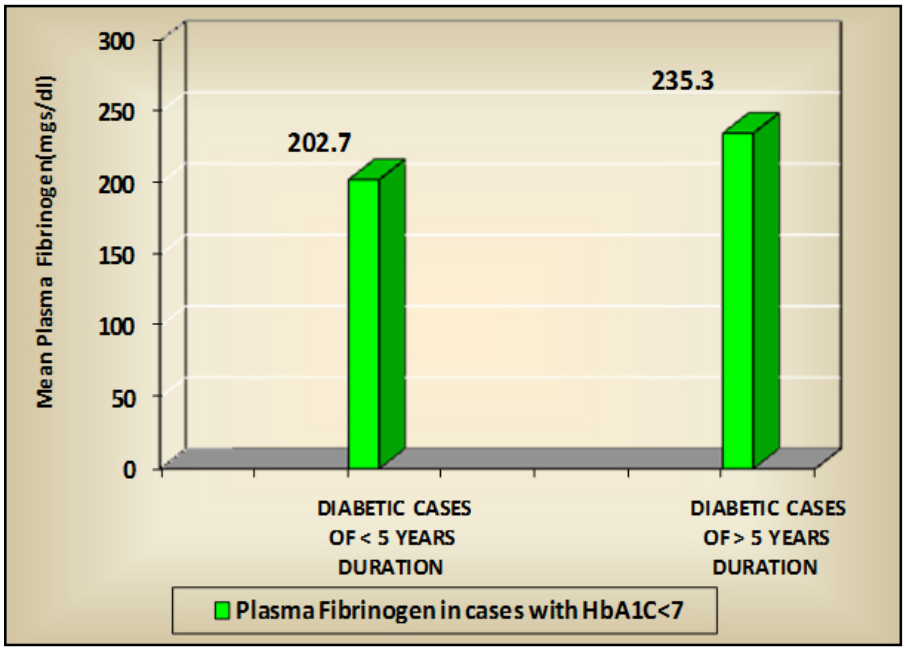


TABLE 5: COMPARISION OF PLASMA FIBRINOGEN LEVEL BETWEEN GROUP IB1 AND GROUP IB2

Group	Plasma Fibrinogen (mgs / dl)	
	Mean	**SD**
Group IB1	315.9	34.7
Group IB2	341.4	92.0
'p'	0.3264 Not significant	

Plasma fibrinogen level increased in patients with more than 5 years duration than in patients with less than 5 years duration.

FIGURE 5:

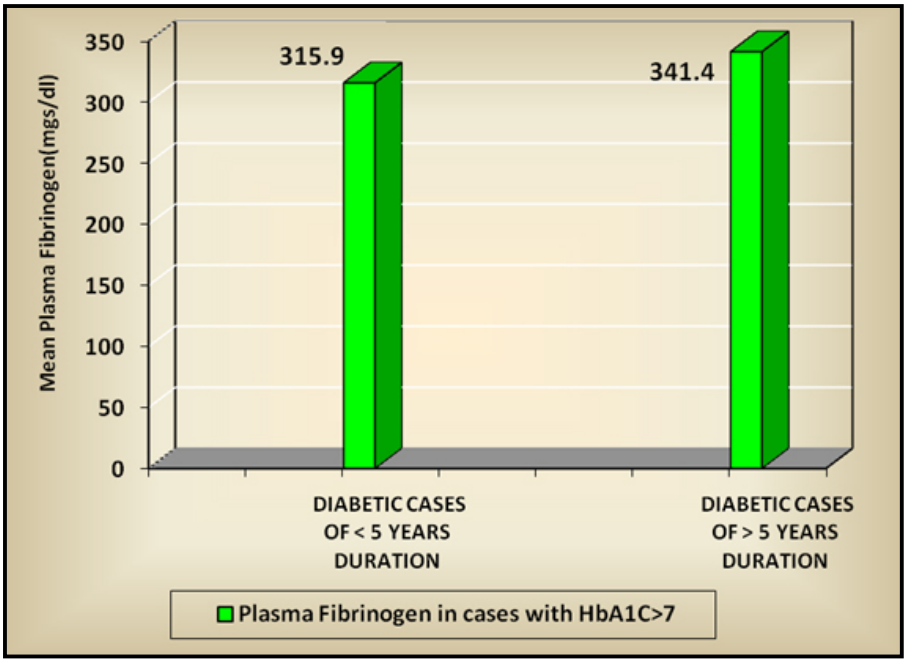


TABLE 6 : COMPARISION OF PLASMA FIBRINOGEN LEVELS BETWEEN DIABETICS OF LESS THAN 5 YEARS AND MORE THAN 5 YEARS DURATION.

Group	Plasma Fibrinogen (mgs / dl)	
	Mean	**SD**
GROUP IA1+IB1	274.2	53.9
GROUP IA2+ IB2	278.1	104.4
'p'	**<0.8534 Not Significant**	

Plasma fibrinogen level increased in patients of more than 5 years duration than in patients of less than 5 years duration, but statistically insignificant.

FIGURE 6:

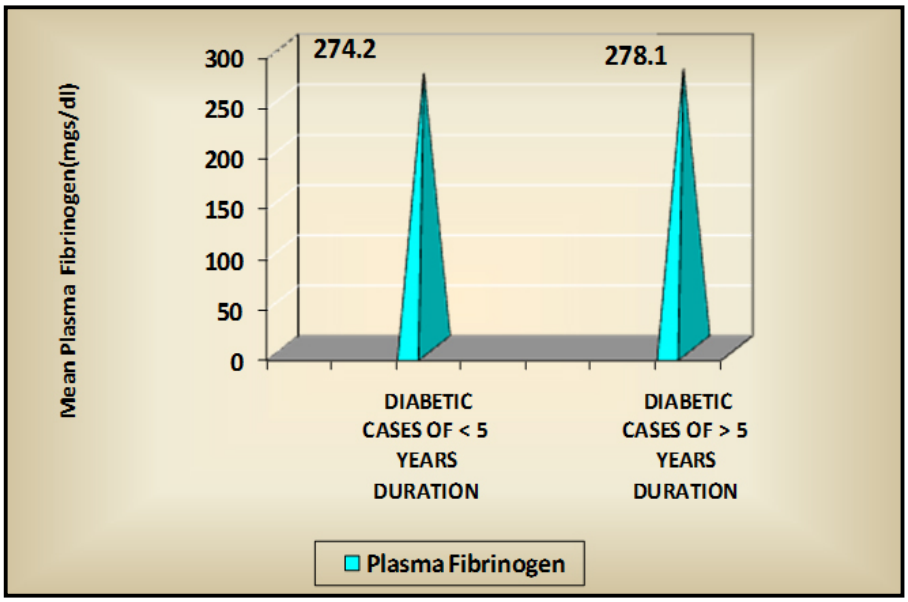


TABLE 7 : COMPARISION OF PLASMA FIBRINOGEN LEVEL BETWEEN GROUP IA AND GROUP II

GROUP	Plasma Fibrinogen (mgs / dl)	
	Mean	**SD**
GROUP IA	217.4	52.0
GROUP II	160.3	49.7
'p'	**<0.0001 Significant**	

Statistically significant difference in plasma fibrinogen was observed between diabetes with HbA1c≤7% and in control group.

FIGURE:7

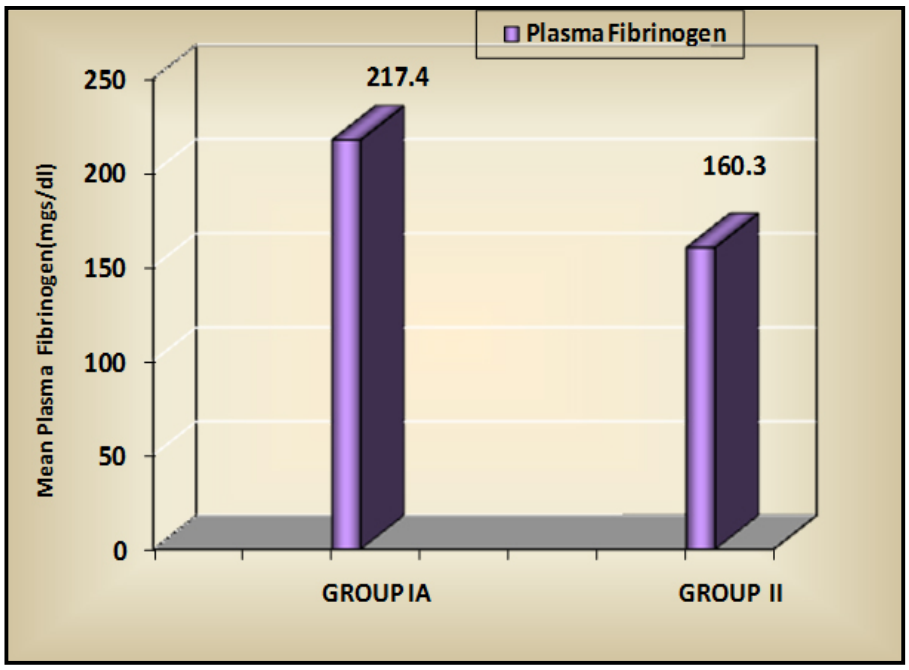


TABLE 8: COMPARISION OF PLASMA FIBRINOGEN LEVELBETWEEN GROUP IB AND GROUP II

GROUP	Plasma Fibrinogen (mgs / dl)	
	Mean	**SD**
GROUP IB	334.2	77.0
GROUP II	160.3	49.7
'p'	**<0.0001 Significant**	

Statistically significant difference in plasma fibrinogen was observed between diabetes with HbA1c>7% and control group.

FIGURE : 8

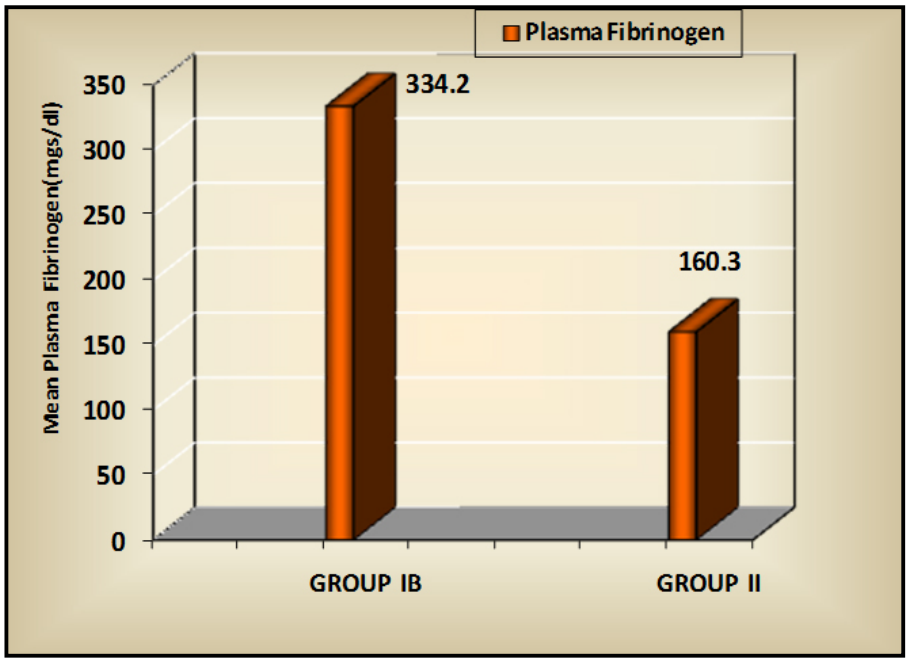
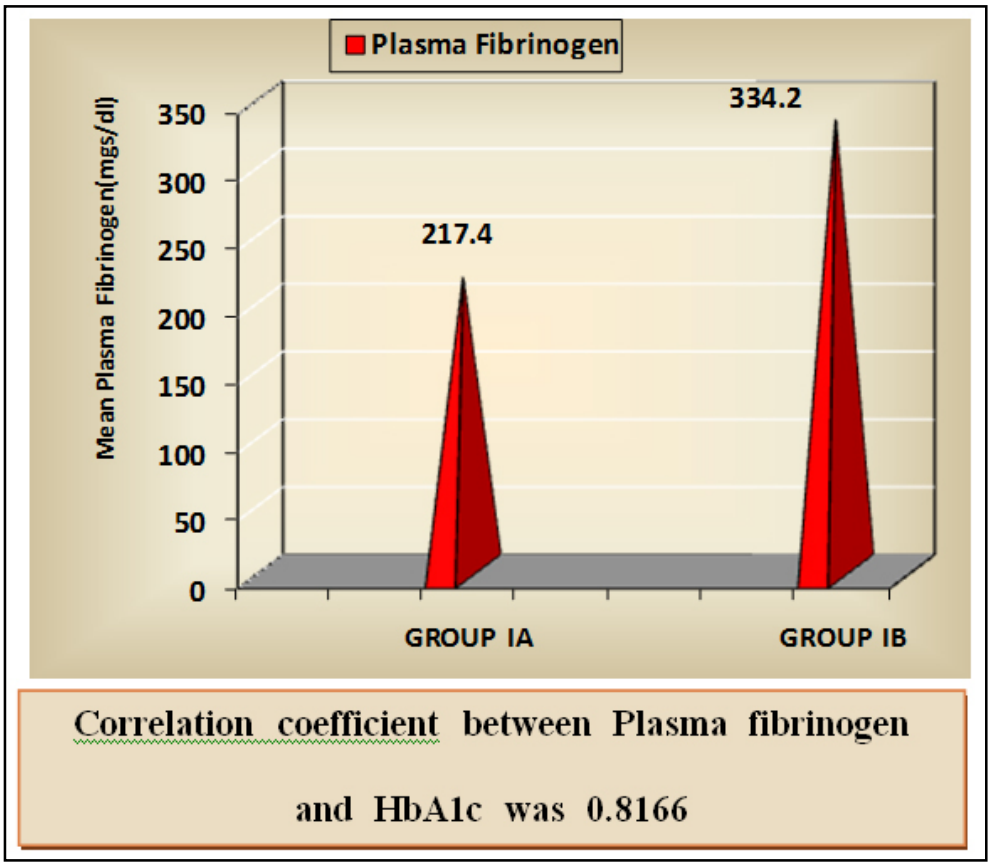


TABLE 9: COMPARISION OF PLASMA FIBRINOGEN LEVEL BETWEEN GROUP IA AND IB

GROUP	Plasma Fibrinogen (mgs / dl)	
	Mean	**SD**
GROUP IA	217	52.0
GROUP IB	334.2	77.0
'p'	**<0.0001 Significant**	

Statistical difference in plasma fibrinogen was observed between diabeticss with HbA1c≤7% and diabetes with HbA1c>7%.

FIGURE :9



DISCUSSION

DISCUSSION

Diabetes mellitus is a global health problem and is expected to affect nearly 330 million people by the year 2030[62]. Cardiovascular diseases like myocardial infarction, angina are common complications of diabetes mellitus. These cardiovascular complications accounting for nearly 75% of death due to thrombosis in diabetes[72].

Traditional risk factors like systemic hypertension, smoking, obesity and dyslipidemia have not been fully responsible for cardiovascular diseases associated morbidity and mortality in diabetes [13].

Altered hemostasis due to hypercoagulable state is implicated as an important risk factor responsible for cardiovascular complications in diabetes. Among the hemostatic factors, fibrinogen has been involved in the pathogenesis of atherosclerosis and considered as an independent risk factor for cardiovascular complications [66].

In the present study, 75 type II diabetes mellitus patients were taken as study group and 75 age and sex matched healthy individuals taken as control group. This study shows that, mean plasma fibrinogen level was significantly increased in the study group when compared to control group. In the study group mean plasma fibrinogen level was 276.6 ± 87.9 mg/dl, whereas in the control group it was 160.3 ± 47mg/dl. These findings were consistent with the study results of Archana Sachin Bembde[68], Nizar M.abderurahman et al[67], Taj Muhammad khan et al[66], Ying zhao et al[71], Ritu Madan et al[72], DR Kafle et al[69], Bruno G et al[65], Venkataramana. G et al[73].

A cross sectional study done among 1574 type II diabetes mellitus patients by Bruno G et al observed the high prevalence of hyperfibrinogenemia in diabetes and fibrinogen level was significantly associated with HbA1c value [65].

Nizar and Elshazali described that mean plasma fibrinogen level was increased significantly in Sudanese diabetic patients but it was in the higher limit of normal range (3.96±0.92)[67].

Another study was done by Ritu Madan et al[72] aimed to know the coagulation status in diabetic patients. They found that, plasma fibrinogen levels were increased in diabetic patients. Other coagulation factors like factor VII, factor VIII, vWF, factor XI, factor XII, Kallikrein, and thrombin antithrombin complexes are also increased in diabetes. They said that, this Procoagulant state is responsible for the occurrence of macrovascular as well as microvascular complications in type II diabetes mellitus.

A cross sectional study done by Taj Muhammad Khan et al to know the association between plasma fibrinogen level and diabetic complications. They have recruited 120 subjects. Among them 40 subjects were controls, 40 subjects were diabetics and 40 subjects were diabetics with complications. They reported that, plasma fibrinogen level and blood viscosity were significantly high in diabetic patients. They have also found significant association between diabetic complications and plasma fibrinogen level[66].

A case control study done on 100 diabetic patients by A. S. Bembde aimed to know the level of plasma fibrinogen in type II diabetes mellitus. They have reported that, Plasma fibrinogen

level was significantly increased in type II diabetic patients when compared to Control group. They said that, existence of prothrombotic state in diabetes was associated with increased plasma fibrinogen level[68].

Various mechanisms were put forward regarding increased plasma fibrinogen level in type II diabetes. The possible mechanisms are,

Low grade chronic systemic inflammation present in type II diabetes is associated with increased synthesis of acute phase reactant proteins by the liver[77].

In an experimental study done in rats showed that, insulin deficiency causes decreased albumin mRNA concentration and decreased albumin synthesis, whereas fibrinogen synthesis was not affected[78]. Similar study done in human diabetic patients reported that insulin deficiency causes 50% increase in fibrinogen synthesis and 29% decrease in albumin synthesis. They said that, this altered protein synthesis is due to insulin deficiency associated metabolic stress on liver[18]. This increased fibrinogen production not balanced by clearance mechanism leads onto elevated fibrinogen in the plasma[76].

Another mechanism is that, advanced glycation end products produced by the non enzymatic glycosylation of intracellular and extracellular proteins induced endothelial dysfunction which leads onto increased amount of clotting factors in the blood. Activation of coagulation pathway results in increased production of thrombin and fibrin degradation products, which in turn enhances fibrinogen production by the liver [71,72].

In the present study, mean plasma fibrinogen level was increased in diabetic patients of more than 5 years duration. Though the fibrinogen level increased, it was statistically insignificant and indicates that duration of diabetes have a less impact on fibrinogen level. This increased level of fibrinogen might be due to pronounced decrement in beta cell activity with increase in insulin resistance in diabetics with more than 5 years duration [67].

The mean plasma fibrinogen level was increased significantly in study group with HbA1c $\leq$ 7% (271.4±52) while compared to control group (160.3±49.7). Mean plasma fibrinogen level was increased significantly in study group with HbA1c > 7% (334.2±77) while compared to control group (160.3±49.7).

Mean plasma fibrinogen level was increased in Patients with HbA1c > 7% (334.2±77) than in Patients with HbA1c ≤ 7% (271.4±52) and these results were statistically significant. These findings were consistent with Study results of DR Kafle and P Shrestha[69], Binayo sapkota et al[15], Ying Zao et al[71], A.S.Bembde[68], Ogbera and Alfred[79], Nizar and Elshazali[67].

Plasma fibrinogen is positively correlated with glycosylated haemoglobin (r=0.8166). These findings were consistent with the study results of Ogbera and Alfred[79], Ying Zao et al[71], Binayo sapkota et al[15], A.S.Bembde[68], Rodolfo-Guardado-Mendoza et al[76], Antonio ceriello et al[70] .

The possible mechanisms responsible for increased fibrinogen level in patients with HbA1c>7% are,

In patients with HbA1c>7% (Poor glycemic control) lipoprotein a levels are suspected to be increased. This LP(a) contains protein moiety called Apo(a). This Apo(a) protein have a structural similarity with plasminogen, which is a precursor of plasmin molecule and have the ability to bind fibrin molecule.

This linkage prevents the binding of plasminogen and tPA with fibrin and by which Apo(a) prevents the clot lysis and promotes more and more of fibrin deposition at the site of vessel injury[68].

More amount of glycosylated fibrinogen are formed during poor glycemic control. Because of this glycosylation, nature of fibrin clot structure was altered and it contains densely packed thin fibrin fibers with reduced pore size. These glycosylated fibrinogen are more resistant to plasmin mediated degradation and also promotes more amount of fibrin deposistion[68,62].

This increased plasma fibrinogen involved in all stages of atherogenesis and considered as important cardiovascular risk factor. Many epidemiological studies have found positive association between plasma fibrinogen level and cardiovascular diseases.

In 1987, The Framingham study reported that there was significant association present between plasma fibrinogen level and coronary artery disease and also added that plasma fibrinogen level was significantly associated with other cardiovascular risk factors like systemic hypertension, obesity,

smoking and diabetes mellitus. This study considers increased plasma fibrinogen level as a predictor of cardiovascular diseases and they have recommended that plasma fibrinogen measurement should be included in the cardiovascular risk profile[38].

Northwick park heart study, analysed the involvement of thrombotic factors in the development of coronary artery diseases. They had investigated 1511 male subjects of age between 40-64 years and found the association between Coronary artery disease and factor VII activity, fibrinogen [39].

Cross sectional study done by Luciana & his co-workers showed that, plasma fibrinogen level was significantly elevated in patients with coronary artery disease while comparing with that of normal subjects. Plasma fibrinogen level was also well correlated with the severity of coronary artery disease [15].

Plasma fibrinogen level was significantly increased in diabetic patients with complications than in diabetic patients without complications[66].

Increased plasma fibrinogen is considered as independent cardiovascular risk factor. In a cross sectional study done on type II diabetics found a strong association between silent

myocardial ischemia and plasma fibrinogen level. It has been suggested that plasma fibrinogen should be added in the cardiovascular risk profile in diabetic patients[76].

SUMMARY

SUMMARY

- Plasma fibrinogen level was compared between Type II diabetic patients and controls.
- Plasma fibrinogen level was significantly increased in diabetic patients compared to controls.
- Plasma fibrinogen level was increased in diabetic patients with more than 5 years duration of diabetes.
- Plasma fibrinogen level was significantly increased in patients who had HbA1c > 7% .
- Plasma Fibrinogen level was positively correlated with HbA1c level.

CONCLUSION

CONCLUSION

Fibrinogen is an important plasma protein involved in the blood coagulation mechanism. Fibrinogen is converted into insoluble fibrin in the injured blood vessel and forms the definite blood clot, which seals off the injured vessel wall effectively.

HbA1c is formed by binding of glucose with β chain of HbA at its amino terminal valine. The life span of RBC is about 120 days, during which haemoglobin gets attached with glucose molecule to form glycosylated haemoglobin. In diabetes Mellitus patients with poor glycemic control, the quantity of glycosylated haemoglobin is in increased amount when compared to patients with good glycemic control.

In the present study , increased plasma fibrinogen level was observed in diabetic patients having HbA1c of more than 7%. It could be due to insulin deficiency associated metabolic stress, advanced glycation end products mediated endothelial

injury, chronic systemic inflammation, Apoprotein - a and increased amount of glycosylated fibrinogen.

This elevated plasma fibrinogen along with other blood clotting factors produce hypercoagulable state in diabetes mellitus. Increased plasma fibrinogen alone also involved in all stages of atherogenesis and considered as an independent cardiovascular risk factor.

The present study also observed decreased level of plasma fibrinogen in patients with HbA1c <7% when compared to patients with HbA1c >7%. It states that, good glycemic control in diabetic patients is necessary to reduce the risk of cardiovascular complications.

Hence present study concludes that, measurement of plasma fibrinogen is considered to be an important tool to assess cardiovascular risk in type II diabetic patients.

LIMITATIONS

The present study considers plasma fibrinogen is an important tool for assessing the cardiovascular risk in type II diabetes mellitus. Large sample size and follow up studies would be of great value.

Measurement of plasma fibrinogen along with other cardiovascular risk factors in diabetic patients will be needed to demonstrate the excess risk of cardiovascular disease associated mortality.

FUTURE STUDY PLAN

The current study is of public health importance as it suggests that, plasma fibrinogen should be added in the screening tool to assess cardiovascular risk status in type II diabetics.

Further followup studies are needed to determine whether good glycemic control would reverse the plasma fibrinogen level and associated cardiovascular risk.

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ANNEXURES

CONSENT FORM

Dr.S.Bhuvaneswari, Post Graduate student in the Department of Physiology, Coimbatore Medical College is studying the "**Correlation between Plasma Fibrinogen Level and Glycosylated Hemoglobin in Type II Diabetes Mellitus Patients"**. Estimation of random blood sugar, plasma fibrinogen, HbA1c were explained to me clearly.

I hereby give my consent to participate in this study.The data obtained herein may be used for research and publication.

Name :

Place :

Signature :

PROFORMA

Name:

Age :

Sex:

Occupation:

Address: Mobile No:

Duration of Diabetes:

History of Myocardial infarction/Stroke/Neuropathy/Nephropathy/peripheral vascular disease

History of Systemic hypertension/ chronic Liver disease/Recent surgery.

H/O drug intake

Smoker/Non smoker

Menstrual History

Height: **Weight:** **BMI:**

Blood Pressure: mm/Hg

Systemic Examination

Respiratorysytem

Cardiovascular system

Central nervous system

Investigation:

Plasma fibrinogen (mg/dl)

HbA1c (%)

Random blood sugar (mg/dl)

MASTER CHART

MASTER CHART I - STUDY GROUP

S.No	Diabetics with HbA1c <7%						duration of diabetes	HbA1c in %	fibrinogen in mgs/dl
	age	sex	BP	weight	height	BMI			
1	46	F	112/80	55	148	25	<5	5.8	218
2	41	M	124/76	62	155	25.8	<5	5.4	173
3	55	F	104/70	53	145	25.2	<5	6.9	210
4	47	F	114/70	53	146	25.2	<5	6.5	268
5	40	F	102/74	55	152	23.9	<5	5.4	168
6	45	F	116/72	56	148	25.5	<5	5.9	235
7	40	F	120/80	60	155	25	<5	5.9	200
8	45	F	96/70	48	156	19.7	<5	6.5	274
9	40	F	104/72	55	146	26.1	<5	6.0	256
10	46	M	120/80	72	170	24.9	<5	6.5	268
11	55	M	114/72	65	160	25.3	<5	6.9	290
12	42	F	110/70	59	156	24.2	<5	6.2	239
13	60	M	110/80	70	163	26.3	<5	6.9	225
14	52	M	112/72	70	162	26.3	<5	6	242
15	44	M	108/78	70	172	23.66	<5	6.4	263
16	52	F	102/74	52	150	23	>5	5.2	112
17	51	M	110/70	63	156	25.8	>5	6.5	283
18	56	M	120/78	68	162	25.9	>5	6.7	265
19	55	M	116/72	65	161	25	>5	5.8	198
20	47	F	106/70	52	156	21.3	>5	5.9	147
21	42	F	100/70	52	153	22.2	>5	5.9	159
22	45	F	98/72	68	162	25.9	>5	6.5	290
23	54	F	112/72	52	146	24.3	>5	6.4	235
24	44	M	114/76	59	160	23	>5	6.8	269
25	50	M	102/74	58	156	23.8	>5	6.1	260
26	53	F	120/80	57	150	25.3	>5	6.4	253
27	45	M	116/70	69	163	25.9	>5	6.7	272
28	60	F	106/70	58	155	24	>5	5.8	142
29	42	M	98/70	67	168	23.7	>5	6.7	240
30	53	M	110/80	72	163	27.1	>5	6.1	153
31	58	M	112/70	65	158	26	>5	5.9	193
32	43	F	120/82	56	150	24.8	>5	6.3	156
33	49	F	114/68	48	150	21.3	>5	6.8	174
34	45	M	106/70	63	156	25.8	>5	7	259
35	52	F	112/70	59	152	25.5	>5	6.2	145
36	47	M	108/70	67	160	26	>5	6.5	124
37	40	F	110/74	58	155	24	>5	5.9	186

BMI - BODY MASS INDEX

BP - BLOOD PRESSURE

HbA1c - HEMOGLOBIN A1c

RBS - RANDOM BLOOD SUGAR

MASTER CHART 2 - STUDY GROUP

S.No	Diabetics with HbA1c >7%						duration of diabetes	HbA1c in %	fibrinogen in mgs/dl
	age	sex	BP	weight	height	BMI			
38	53	F	120/80	55	152	23.8	<5	8.1	320
39	47	M	120/80	62	156	25.4	<5	7.3	272
40	45	M	112/74	69	162	26.2	<5	10.6	360
41	57	F	110/70	65	160	25.3	<5	8.4	326
42	43	M	126/70	70	163	26.35	<5	7.8	312
43	55	F	106/70	60	158	24	<5	12.6	280
44	58	F	110/80	63	153	26.9	<5	10.7	372
45	46	M	116/70	68	158	27.2	<5	7.8	290
46	49	F	108/74	58	146	27.2	<5	9.9	315
47	45	M	114/70	65	163	24.4	<5	10.0	354
48	44	F	114/64	58	151	25.4	<5	8.5	320
49	53	M	122/76	73	168	25.8	<5	7.4	263
50	55	F	124/70	54	158	21.6	<5	10.8	353
51	41	M	110/74	62	157	25	<5	8.0	285
52	43	M	110/70	59	162	22.4	>5	13.0	386
53	46	M	116/70	66	160	25.7	>5	7.7	265
54	49	F	120/76	58	155	24.1	>5	8.1	298
55	53	M	110/80	68	162	25.9	>5	12.1	375
56	40	F	106/70	63	161	24.3	>5	8.5	270
57	46	M	112/70	68	170	23.5	>5	11.4	295
58	54	M	116/74	68	158	27.2	>5	11.6	322
59	50	M	110/78	63	165	23	>5	13.3	430
60	52	F	106/72	65	153	26.9	>5	13.5	520
61	47	M	108/74	62	155	25.8	>5	8.4	245
62	41	M	110/80	70	165	25.7	>5	12.9	310
63	60	F	120/76	60	158	24	>5	10.7	339
64	49	F	120/70	65	155	27	>5	10.2	337
65	45	F	114/72	63	157	25.5	>5	9.3	212
66	48	F	124/80	60	151	26.3	>5	10.1	305
67	44	M	106/76	68	165	24.9	>5	8.2	295
68	49	F	104/70	60	153	25.6	>5	7.8	247
69	52	F	112/70	57	158	22.8	>5	13.0	552
70	56	F	116/70	63	156	25.8	>5	13.5	568
71	43	M	112/70	65	167	23.3	>5	11.5	365
72	56	M	108/76	68	162	25.9	>5	12.5	370
73	51	F	120/80	59	153	25.2	>5	8.5	320
74	42	F	120/80	54	150	24	>5	8.5	345
75	46	F	122/76	60	152	25.9	>5	11.6	305

BMI - BODY MASS INDEX

BP - BLOOD PRESSURE

HbA1c - HEMOGLOBIN A1c

RBS - RANDOM BLOOD SUGAR

MASTER CHART 3 - CONTROL GROUP

S.No	Age	Sex	BP	Weight	Height	BMI	RBS	fibrinogen
76	40	M	106/70	62	158	27.5	112	167
77	46	M	110/70	70	164	28	124	93
78	52	M	120/80	65	162	25	140	96
79	43	F	114/74	55	150	24	98	200
80	46	F	112/72	48	152	21.3	117	135
81	48	F	114/80	55	148	24	114	139
82	42	F	116/70	57	150	25.3	136	105
83	51	M	114/80	65	168	23	145	200
84	55	F	120/76	64	162	25	116	142
85	55	F	114/70	60	156	26.6	124	102
86	54	F	120/80	59	160	23	116	114
87	44	F	124/80	58	156	24	118	118
88	42	M	116/80	57	150	25.3	136	78
89	41	F	114/74	69	163	26.9	122	112
90	53	F	112/80	55	155	24.4	124	142
91	56	M	116/80	60	168	21.4	102	133
92	55	M	112/80	72	163	28	96	176
93	51	F	114/74	65	158	26	120	149
94	43	M	116/70	56	150	24.8	115	195
95	42	M	120/82	48	150	21.3	106	147
96	46	M	124/80	63	156	26.2	102	112
97	41	F	120/80	59	152	26.2	116	128
98	45	M	122/80	67	155	27.9	118	126
100	49	M	124/80	58	155	25.7	94	145
101	52	M	112/70	55	152	23.9	86	194
103	53	F	120/76	62	156	25.8	110	173
104	55	F	126/80	69	162	27.6	100	187
105	41	F	120/80	65	160	25.3	98	108
106	43	F	112/70	70	163	27.3	126	153
107	46	M	114/74	65	158	25.39	132	104
108	49	M	112/72	63	153	28	145	159
109	53	F	114/80	68	156	29.56	112	118
110	40	M	116/70	52	146	24.7	124	91
111	46	F	114/80	65	157	25	140	172
112	54	M	120/76	58	151	25.7	98	121
113	50	M	110/80	70	168	25	117	93
114	52	M	120/76	54	158	21	114	124
115	47	F	120/70	62	157	24.2	136	186
116	41	F	114/72	57	162	22.6	145	148
117	60	M	124/80	66	160	25.7	116	79
118	49	F	106/76	58	155	24	124	212
119	45	M	104/70	68	162	26.5	116	189
120	48	F	112/70	55	148	25	118	232
121	44	M	116/70	62	155	25.8	136	220

122	46	M	112/70	56	145	25.4	122	270
123	41	M	108/76	53	146	23.5	106	195
124	55	F	120/80	55	152	24.4	102	215
125	47	F	120/80	58	148	25.7	116	125
126	40	M	122/76	60	155	25	118	160
127	45	F	110/74	52	156	21.6	94	135
128	40	M	110/70	55	146	25	86	105
129	45	F	116/70	72	170	24.8	110	226
130	65	M	120/76	65	160	25.3	100	163
131	46	M	110/80	59	156	24.5	98	145
132	55	F	106/70	70	158	29.1	126	176
133	64	F	112/70	73	162	32.4	132	153
134	60	M	116/74	55	148	24.4	145	155
135	52	F	110/78	62	155	25.8	112	98
136	44	M	106/72	56	145	25.4	124	168
137	52	M	110/74	53	146	24	140	260
138	51	F	110/70	55	152	24.4	106	114
139	56	M	116/70	58	148	26.3	102	126
140	55	F	120/76	60	155	25	116	236
141	47	F	110/80	52	156	21.6	118	212
142	42	M	106/70	55	146	25	94	272
143	45	F	112/70	72	170	24.8	86	182
144	54	M	116/74	65	160	25.3	110	248
145	44	F	110/78	59	156	24.5	100	232
146	60	M	106/70	70	158	29	98	250
147	51	F	110/74	73	162	28.5	126	230
148	42	M	110/70	55	148	25	132	125
149	40	M	116/70	62	155	25.8	145	195
150	40	M	120/76	56	145	24.4	112	215

BMI - BODY MASS INDEX

BP - BLOOD PRESSURE

RBS - RANDOM BLOOD SUGAR